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Coupling the Cell cycle and the Circadian Cycle

Laurence Calzone * , Sylvain Soliman *

Thèmes SYM et BIO — Systèmes symboliques et Systèmes biologiques
Projet Contraintes

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Abstract: Cancer treatments based on the administration of medicines at different times of the day have been shown to be more efficient against malign cells and less damaging towards healthy ones. These results might be related to the recent discovery of links between the circadian clock, (controlled by the light/dark cycle of a day), and the cell cycle. However, if many models have been developed to describe both of these cycles, to our knowledge none has described a real interaction between them. We will try to establish a relation at a molecular level and we will then study the conditions of entrainment in period of these cycles with the modeling environment BIOCHAM. In other words, we will search how and where in the parameter space of our model the two cycles get synchronized. This technical report will insist on the conditions of entrainment of the cell cycle by the circadian cycle via a common protein kinase WEE1.

Key-words: Cell cycle, circadian clock, mathematical model, entrainment

* Projet Contraintes – Name.Firstname@inria.fr

Couplage des cycles cellulaire et circadien

Résumé : Des expériences récentes montrent que le traitement des cancers par des chimiothérapies peut être sensiblement plus efficace et moins traumatisant si les cytotoxiques sont administrés à différents moments de la journée. Cette constatation est à mettre en parallèle avec la découverte récente de liens entre l'horloge circadienne (contrôlée par l'alternance lumière/obscurité) et le cycle cellulaire. Cependant, si des modèles de ces deux cycles ont déjà été développés, aucun ne décrit réellement, à notre connaissance, l'interaction entre eux. Nous proposons d'établir une telle relation au niveau moléculaire puis étudions les conditions d'entrainement en période du cycle cellulaire. En d'autres termes, nous allons, à l'aide de l'environnement de modélisation BIOCHAM, rechercher dans l'espace des paramètres les régions de synchronisation des deux cycles. Ce rapport se focalise en particulier sur les conditions d'entrainement du cycle cellulaire par le cycle circadien à travers la kinase WEE1.

Mots-clés : Cycle cellulaire, horloge circadienne, modélisation mathématique, entrainement

1 Introduction

Cancer chronotherapies are more and more used in cancer treatments, based on the administration of medicines at different times of the day. They have been shown to be both more efficient against malign cells and less damaging towards healthy ones [8, 6]. These results might be related to the recent discovery of links between the circadian clock, (controlled by the light/dark cycle of a day), and the cell cycle [7, 1, 10]. However, if many models have been developed to describe both of these cycles, none has described a real interaction between them. Based on the coupling of two numerical models, one for the circadian cycle directly adapted from [5], and a generic one for the cell cycle (inspired by [9] and then modified to fit our needs), we will try to establish a relation at a bio-molecular level. Then, using the modeling environment BIOCHAM [3, 2] we will study the conditions of entrainment in period of these cycles, in other words, how and where in the parameter space of our model the two cycles get synchronized. Though some other links have recently been hypothesized, this technical report will insist on the conditions of entrainment of the cell cycle by the circadian cycle via a common protein kinase WEE1.

1.1 A mammalian circadian cycle

In many organisms, spontaneous oscillations with a period close to 24 hours have been observed. A complex network of proteins, sensitive to light, is organized to maintain these oscillations at this period. In mammalian cells, two major proteins are transcribed in a circadian manner, CLOCK and BMAL1 which bind to form a heterodimer responsible for the transcription of *per* (period) and *cry* (cryptochrome). The two newly-formed proteins then bind and as soon as the activity of the complex reaches a threshold, PER/CRY associates with the complex CLOCK/BMAL1 to inhibit its activity and therefore the transcription of the two proteins PER and CRY. From this negative feedback loop, oscillations are born and maintained. A model proposed by Leloup and Goldbeter on mammalian cell cycle describes in more detail the negative feedback loops involved in this mechanism [5].

1.2 Experimental evidence of links between the circadian and cell cycles

Matsuo and colleagues [7] observed regenerating liver cells and measured the expression of proteins involved in the cell cycle mechanism at different hours of the day, especially the WEE1 kinase and its target, the cyclin-dependent kinase *cdc2*. The WEE1 kinase seems to be good a candidate to establish a link between the cell and circadian cycles during the G2-M transition (see next section for more details). Indeed, mutations of circadian genes have a direct impact on *wee1* mRNA and protein patterns. For example, a deficient *cry* mutant exhibits high levels of *wee1* mRNA throughout regeneration [7], suggesting that WEE1 is regulated by some components of the circadian cycle. The change in CDC2 activity might be a consequence of the change in WEE1. The *wee1* gene promoter is believed to be activated by the complex CLOCK/BMAL1 and inhibited by CRY.

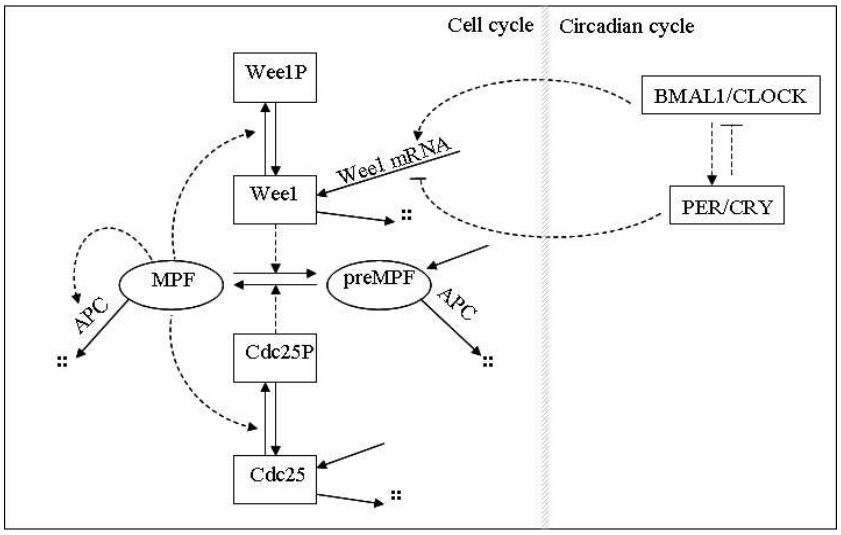

Figure 1: Linking the circadian and the cell cycles via WEE1

There are of course other links by which the circadian rhythm influences the cell cycle. It has been, for instance, reported that PER2 regulates the transcription of *c-myc*, and in a moderate way that of *mdm2* [4], links through *tim* (timeless) are also hypothesized [1, 10]. However, the precise mechanisms involved are still not completely clear and require a very detailed description of the cell cycle, including DNA-damage response, precise E-box related transcription mechanics, distinction between PER1, PER2 and PER3, etc. In this report, we will thus restrict our study to the link established through WEE1, which will allow us to rely on a simpler and more generic model of the cell cycle, focused on mitosis. As detailed in the discussion, a more complete model is currently under study.

1.3 A simplified generic cell cycle

The cell cycle of somatic cells is composed of four phases: DNA replication (S phase) and chromosome segregation (M phase) separated by two gap phases (G1 and G2). For our purpose, the model describing a generic cell cycle will focus on the G2-M transition during which the protein WEE1 plays a significant role. The cell cycle will thus be divided in two different phases, the G1-S-G2 and M phases.

At the center of the cell cycle regulation, there is a group of proteins, the cyclin-dependent kinases. The cyclin-dependent kinases are composed of two subunits, a kinase *cdc2*, and a cyclin partner which determines the specificity of the complex. The activity of the complexes

leads to different actions in the cell cycle. Indeed, according to the cyclin, the complex can trigger DNA replication, or entry into mitosis, etc ...

The major one is a B-type cyclin. The complex CDC2/cyclinB appears in two forms, an active form called MPF (for M-phase Promoting Factor), and an inactive form referred to as preMPF. There are several ways to regulate its activity. One of them involves the kinase WEE1 and the phosphatase CDC25. MPF is phosphorylated and inactivated by WEE1, and dephosphorylated and activated by CDC25. Both the kinase and phosphatase activities are themselves regulated by MPF, respectively inactivated and activated by the complex.

The proteins chosen to illustrate the cell cycle presented in this report are MPF, preMPF, the degradation factor of the cyclins, APC, the WEE1 kinase and the CDC25 phosphatase (Figure 1). Early in the cycle, MPF is kept inactive because the cyclin is not synthesized and WEE1 is present. As the cyclin is slowly synthesized, MPF activates and reaches a threshold that both inactivates WEE1 and activates CDC25 which maintains MPF in its active state. The cell enters mitosis. With a short delay, APC is activated and degrades the cyclin component of MPF. The cell exits mitosis and repeats its cycle. The model is composed of two positive feedback loops (CDC25 activates MPF which in turn activates CDC25, and WEE1 inactivates MPF which in turn inactivates WEE1) and a negative feedback loop (MPF activates APC through an intermediary enzyme X and APC degrades the cyclin component of the complex MPF) (Figure 2).

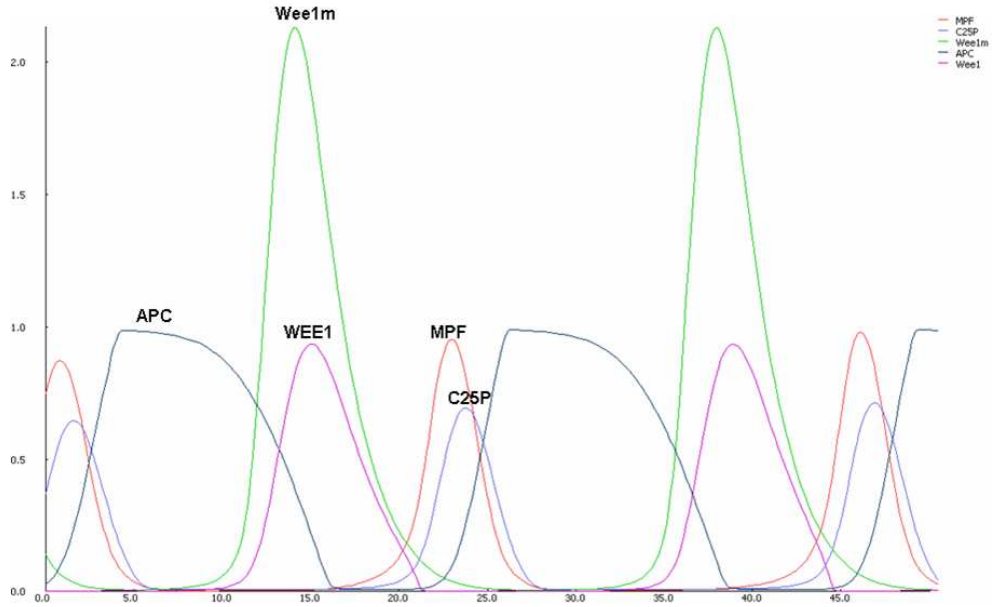

Figure 2: Temporal simulation of a generic cell cycle

The biochemical reactions describing the system are written in the BIOCHAM environment [3, 2] from which nonlinear ordinary differential equations are deduced (Table 1) with the corresponding parameters (Table 2) that describe the aforementioned behavior. The parameters are chosen such that the cycle has an autonomous period of oscillations of 22.4h.

$$
\begin{aligned}
\frac{d[preMPF]}{dt} &= ksmpf + kimpf \cdot [Wee1] \cdot [MPF] - kampf \cdot [C25P] \cdot [preMPF] \\
&\quad -kdmpf \cdot [APC] \cdot [preMPF] - kdmpfp \cdot [preMPF] \\
\frac{d[MPF]}{dt} &= kampf \cdot [C25P] \cdot [preMPF] - kimpf \cdot [Wee1] \cdot [MPF] \\
&\quad -kdmpf \cdot [APC] \cdot [MPF] - kdmpfp \cdot [MPF] \\
\frac{d[C25]}{dt} &= \frac{Vic \cdot [C25P]}{Kic25 + [C25P]} + ks25 - kd25 \cdot [C25] - \frac{Vapc + Vac \cdot [MPF] \cdot [C25]}{Kac25 + [C25]} \\
\frac{d[C25P]}{dt} &= \frac{Vapc + Vac \cdot [MPF] \cdot [C25]}{Kac25 + [C25]} - kd25 \cdot [C25P] - \frac{Vic \cdot [C25P]}{Kic25 + [C25P]} \\
\frac{d[Wee1]}{dt} &= \frac{Viw \cdot [Wee1P]}{Kiw + [Wee1P]} + kswee \cdot [Wee1m] \\
&\quad -kdwee \cdot [Wee1] - \frac{Vapw + Vaw \cdot [MPF] \cdot [Wee1]}{Kaw + [Wee1]} \\
\frac{d[Wee1P]}{dt} &= \frac{Vapw + Vaw \cdot [MPF] \cdot [Wee1]}{Kaw + [Wee1]} - kdwee \cdot [Wee1P] - \frac{Viw \cdot [Wee1P]}{Kiw + [Wee1P]} \\
\frac{d[APC]}{dt} &= \frac{kaapcp + kaapc \cdot [X] \cdot [APCi]}{Kapc + [APCi]} - \frac{kiapc \cdot [APC]}{Kapc + [APC]} \\
\frac{d[X]}{dt} &= ksx \cdot [MPF] - kdx \cdot [X] \\
\frac{d[Wee1m]}{dt} &= \frac{ksweemp + ksweem \cdot [BN]}{Kweem + kwpcn \cdot [PCN]} - kdweem \cdot [Wee1m] \\
\frac{d[APCi]}{dt} &= \frac{kiapc \cdot [APC]}{Kapc + [APC]} - \frac{kaapcp + kaapc \cdot [X] \cdot [APCi]}{Kapc + [APCi]}
\end{aligned}
$$

Table 1: Equations of the cell cycle model

2 Results

2.1 Linking the two cycles

The two models describing the cell and circadian cycles are linked through the transcription of WEE1. An equation for *wee1* mRNA, called Wee1m, is added to the set of equations of

ksmpf=0.3	*kdmpf'*=0.25	*kdmpf*=2	*kimpf*=1	*kampf*=5
ks25=1	*kd25*=1	*Vic*=0.2	*Vac'*=0.1	*Vac*=1
kswee=0.5	*kdwee*=1	*Viw*=0.2	*Vaw'*=0.1	*Vaw*=1
ksx=0.3	*kdx*=0.1	*kaapc*=1	*kiapc*=0.5	
Kac=0.01	*Kic*=0.01	*Kaw*=0.005	*Kiw*=0.005	*Kapc*=0.01
ksweem'=0.001	*ksweem*=0.5	*kdweem*=1	*Kweem*=0.2	*kwpcn*=5

Table 2: Parameters

the cell cycle in Table 1. In the model of the cell cycle alone, *wee1* mRNA was a parameter equal to 1. In the coupled model, the equation for Wee1m is as follows:

$$\frac{d[Wee1m]}{dt} = \frac{ksweemp + ksweem \cdot [BN]}{Kweem + kwpcn \cdot [PCN]} - kdweem \cdot [Wee1m]$$

where the production of Wee1m is a function of the nuclear form of the complex BMAL1/CLOCK (BN) and the unphosphorylated nuclear form of the complex PER/CRY (PCN). Note that a mutation of *cry* affects negatively BMAL1/CLOCK and by extension the mRNA of *wee1*. The negative effect of PCN (nuclear PER/CRY in the model) does not need to appear in the equation since it already has an influence on the activity of BMAL1/CLOCK. However, we choose to include it in the equation anyway to insist on the negative effect of CRY. The degradation of *wee1* mRNA is considered to be linear here. As for WEE1 protein, its production is proportional to the amount of *wee1* mRNA present in the cell and its degradation is assumed to be linear. The phosphorylation/dephosphorylation terms are not modified from the previous model:

$$\begin{aligned}\frac{d[Wee1]}{dt} &= \frac{Viw \cdot [Wee1P]}{Kiw + [Wee1P]} + kswee \cdot [Wee1m] \\ &\quad -kdwee \cdot [Wee1] - \frac{Vapw + Vaw \cdot [MPF] \cdot [Wee1]}{Kaw + [Wee1]}\end{aligned}$$

Once the equations are written, the parameter values need to be chosen carefully and these choices appear to be less obvious than expected. Several values were tried for the new parameters (*ksweem, kdweem, Kweem* and *kwpcn*) along with the ones that were related to WEE1 (*kswee, kdwee* and *kimpf*) and diverse behaviors were obtained. The full model in BIOCHAM syntax is given in appendix.

2.2 Conditions of entrainment

To find values for which entrainment occurs, the parameter space for each parameter is explored. The values of three parameters appeared to be more significant than others: *ksweem*, *kswee* and *kimpf*. The two parameters *ksweem* and *kswee* both control the level of

the WEE1 protein and show such similarities that in the following discussion, we will only report on *kswee*. The parameter values are varied in a given interval and reveal domains of entrainment reported in Figure 3. The parameters are plotted as a function of the period of three proteins that account for the behavior of the two cycles, BN (BMAL1/CLOCK nuclear) for the circadian cycle, MPF for the cell cycle, and their link, WEE1. For low values of the parameters (region 1), MPF and BN have independent periods of oscillations of 22.4h and 23.85h respectively and no sustained oscillations are observed for WEE1. The reason for the perturbation in WEE1 oscillations in this region is that WEE1 receives simultaneously two influences: from the circadian cycle that controls the transcription of the protein mediated by the circadian transcription factors BMAL1/CLOCK and PER/CRY; and from the cell cycle that controls the activity of the protein via phosphorylation by MPF. WEE1 is produced but as soon as MPF activates, it is inactivated because WEE1 has no or little effect on MPF activation and MPF inhibits WEE1 protein. The two influences operate on WEE1 at different times as they both have different periods, perturbing WEE1 period.

For intermediate values of the parameters (region 2), WEE1 starts to play a more significant role in the cell cycle by inhibiting MPF activity, and as a result, disturbing MPF oscillations. It is only when the parameters reach a high value (either *kimpf*=1.2 or *kswee*=0.4) that the oscillations of MPF become stable again but with a period similar to that of the circadian cycle (region 3) revealing the entrainment of the cell cycle through WEE1 activity (through *kimpf*) or protein level (through *kswee*).

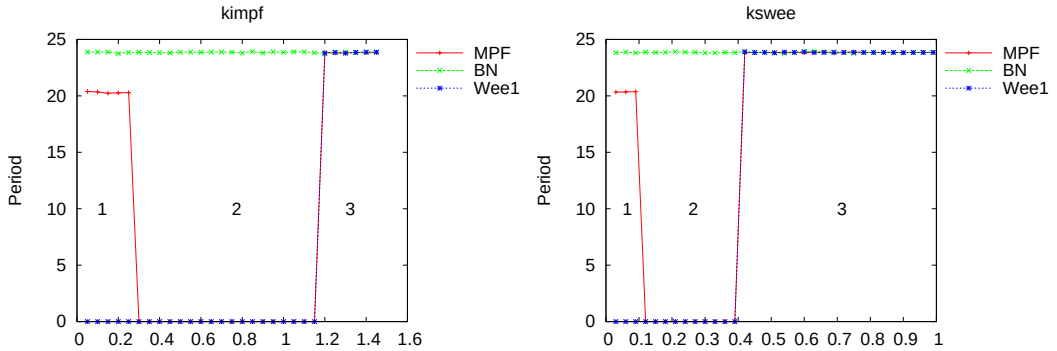

Figure 3: Plot of the period as a function of the parameter *kimpf* from 0.01 to 1.6 and *kswee* from 0.01 to 1. The system shows entrainment for values superior to 1.2 for *kimpf* and 0.4 for *kswee*. For our purposes, constant periods are defined as follows: the last 11 peaks of the simulation over 500 time units show no more than 4% difference in their maxima and the length of the periods. MPF starts with an autonomous period of 22.4h and BN a period of 23.85h. As *kimpf* and *kswee* increase, MPF oscillations (accounting for cell cycle) lose stability and are entrained, along with WEE1, for higher values of the parameter with a period of 23.85h.

The same observations can be made by looking at the trajectories of MPF as a function of BN (Figure 4a) and of MPF as a function of both BN and WEE1 (Figure 4b) for three different values of *kimpf* taken in the three regions. Only when entrainment occurs, does the trajectory follow a single path revealing a limit cycle. From these observations, it can be confirmed that the concentration or the activity of WEE1 seems to be determinant in the entrainment of the cell cycle by the circadian cycle.

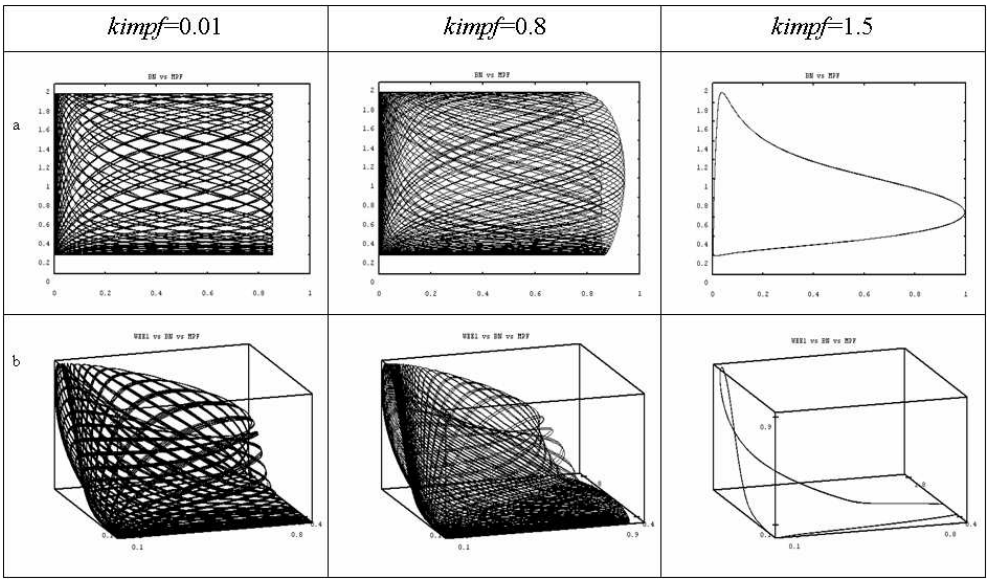

Figure 4: (a) 2-D plots of trajectories of BN as a function of MPF. (b) 3-D plots of trajectories of BN as a function of MPF and WEE1. As *kimpf* increases, the trajectories approach a stable oscillatory regime. For *kimpf*=1.5, BN and MPF show a stable trajectory around a stable limit cycle but with an antiphasic behaviour. For *kimpf*=0.01 and *kimpf*=0.8, the cell cycle (illustrated by MPF) is not entrained by the circadian cycle (illustrated by BN).

However, the study of *kimpf*, the parameter controlling the activity of WEE1 on MPF inactivation, shows that the entrainment does not solely depend on the value of the parameter but more particularly on the ratio *kimpf*/*kampf* since both CDC25 and WEE1 are involved in the positive feedback loops that activate MPF and therefore responsible for the G2-M transition. To investigate this dual effect, the limit of entrainment is measured as the two parameters *kimpf* and *kampf* are varied simultaneously. A linear function of the form: $kampf = 2.44832 \cdot kimpf + 2.0071$ is obtained, the region below the line being the entrainment region (Figure 5).

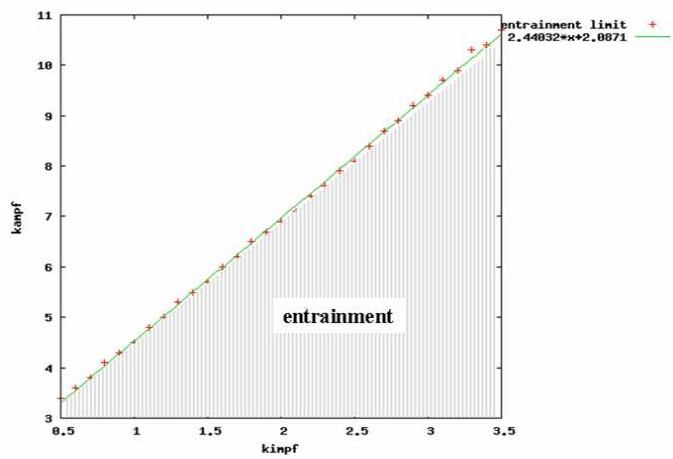

Figure 5: BIOCHAM-generated plot of the entrainment in period of the cell cycle by the circadian cycle for different values of *kimpf* (action of WEE1 on MPF) and *kampf* (action of CDC25 on MPF). The limit of entrainment computed by BIOCHAM (red crosses) is interpolated by the linear function $kampf = 2.44832 \cdot kimpf + 2.0071$ (solid line).

3 Discussion

In this report, we have considered the entrainment of the cell and circadian cycles at the G2-M transition through the WEE1 kinase protein and defined the specific conditions under which this entrainment occurs. We have shown here that WEE1 shares two kinds of influence from both the circadian and the cell cycles. On one hand, experimental data suggest that circadian genes mediate WEE1 transcription and on the other hand, it is believed that MPF regulates WEE1 activity by phosphorylating and inactivating it. Therefore, the timing of the G2-M transition depends not only on the level of WEE1 protein but also on the action of WEE1 on MPF as opposed to that of CDC25.

In the model presented here, since the focus is on the G2-M transition, the coupling of the two cycles only accounts for the entrainment of period but not of phase. However, even though tumor cells keep a period of 24h, they are believed to show a shift of phase. Because of the simplicity of our cell cycle representation, this shift cannot be illustrated in this model. In the future, a more complete model of the four phases of the cell cycle will account for the phase shifts and provide a better analysis of the entrainment conditions. Such a model would allow us to simulate both healthy and cancerous cells and include links to the circadian cycle involving *mdm2* or *c-myc* which might be a good candidate for keeping the period of cancerous cells close to 24h .

3.1 Therapeutic perspectives

As pointed out by the recent research about cancer chronotherapies, circadian rhythms can have a very strong effect on the toxicity and the efficacy of anti-tumor drugs [6]. One possible explanation is that the effect of anti-cancer drugs on a (healthy or tumorous) cell is dependent on the phase of the cell cycle in which that cell lies. Healthy tissues remaining mostly synchronized by the coupling described above, it is possible to drastically reduce the toxicity by injecting the antitumor drugs when they harm as few healthy cells as possible. On the other hand, tumorous cell are either phase-shifted (slow-growing tumors) or not entrained any more (rapidly growing or advanced stage tumors) [8]. Only the first case will really benefit from an increased efficacy of a rhythmic drug injection. However, if it were possible to re- entrain the cells that have lost the 24h period and bring them back to the phase-shifted state of early tumor cells, then even advanced tumors would benefit from an improved efficacy of chronotherapies. The entrainment conditions described in this report suggest that such re-entrainment in period would be possible, improving once again the efficacy/toxicity ratio.

Moreover, even if the mechanism proposed above is incomplete or not fully correct, Mormont and Lévi observed that reinforcing the circadian rhythm (mainly by the rest-activity cycles) improved the survival rate of cancer patients. They conclude that light therapies or sleep management might be used to help in chronotherapies. We propose here a basis for a biochemical alternative to reinforce the effect of the circadian rhythm, and thus enhance the effect of cancer therapies. For example, drugs targeting WEE1 or CDC25 might help modulate the protein's concentration or activity and therefore force the entrainment in period of cancer cells.

Acknowledgements

We would like to thank François Fages for his always thought-provoking remarks and useful questioning about methodology, and Albert Goldbeter for his initial input on the direction to take for this work.

Full model in BIOCHAM syntax

```
% Cell cycle adapted from Qu et al, Biophysical journal

% Parameters

parameter(kd25,1).          parameter(Kiw,0.005).    parameter(kdmpfp,0.25).
parameter(Vapw,0.1).        parameter(kampf,5).      parameter(Kapc,0.01).
parameter(kaapc,1).         parameter(ks25,1).       parameter(ksweemp,0.001).
parameter(kswee,0.5).       parameter(ksweem,0.5).   parameter(kimpf,1.5).
parameter(kdweem,1).        parameter(Vic,0.2).      parameter(Kweem,0.2).
parameter(kiapc,0.5).       parameter(kwpcn,5).      parameter(kdwee,1).
```

```
parameter(Vac,1).          parameter(Viw,0.2).     parameter(Kac25,0.01).
parameter(ksmpf,0.35).     parameter(Kic25,0.01).  parameter(kdx,0.1).
parameter(kdmpf,2).        parameter(Kaw,0.005).   parameter(Vaw,1).
parameter(ksx,0.3).        parameter(Vapc,0.1).

% Reaction rules
% MPF - preMPF
ksmpf                   for _=>preMPF.
kdmpfp*[preMPF]         for preMPF=>_.
kdmpfp*[MPF]            for MPF=>_.
kdmpf*[APC]*[MPF]       for MPF=[APC]=>_.
kdmpf*[APC]*[preMPF]    for preMPF=[APC]=>_.

kampf*[C25P]*[preMPF]   for preMPF=[C25P]=>MPF.
kimpf*[Wee1]*[MPF]      for MPF=[Wee1]=>preMPF.

macro(ratio,kampf/kimpf).

% Wee1
Viw*[Wee1P]/(Kiw+[Wee1P])                    for Wee1P=>Wee1.
(Vapw+Vaw*[MPF])*[Wee1]/(Kaw+[Wee1])         for Wee1=[MPF]=>Wee1P.

(ksweemp+ksweem*[BN])/(Kweem+kwpcn*[PCN])    for _=[BN]=>Wee1m.
kdweem*[Wee1m]                               for Wee1m=>_.

kswee*[Wee1m]  for _=[Wee1m]=>Wee1.
kdwee*[Wee1]   for Wee1=>_.
kdwee*[Wee1P]  for Wee1P=>_.

% Cdc25
Vic*[C25P]/(Kic25+[C25P])              for C25P=>C25.
(Vapc+Vac*[MPF])*[C25]/(Kac25+[C25])   for C25=[MPF]=>C25P.

ks25           for _=>C25.
kd25*[C25]     for C25=>_.
kd25*[C25P]    for C25P=>_.


% APC
ksx*[MPF]     for _=[MPF]=>X.
kdx*[X]      for X=>_.

kaapc*[X]*[APCi]/(Kapc+[APCi])   for APCi=[X]=>APC.
kiapc*[APC]/(Kapc+[APC])         for APC=>APCi.
```

```
% Leloup - Goldbeter PNAS June 2003 Vol. 100 n12 7051-7056

parameter(vsP,1.5).     parameter(kdn,0.01).    parameter(V4PC,0.1).
parameter(KAP,0.7).     parameter(ksP,0.6).     parameter(k8,0.1).
parameter(vmP,1.1).     parameter(V2P,0.3).     parameter(V3PC,0.4).
parameter(KmP,0.31).    parameter(Kdp,0.1).     parameter(k7,0.5).
parameter(kdmp,0.01).   parameter(k4,0.2).      parameter(vdPCC,0.7).
parameter(vsC,1.1).     parameter(V1P,0.4).     parameter(vdPCN,0.7).
parameter(KAC,0.6).     parameter(Kp,0.1).      parameter(ksB,0.12).
parameter(vmC,1).       parameter(k3,0.4).      parameter(V2B,0.1).
parameter(KmC,0.4).     parameter(vdPC,0.7).    parameter(k6,0.2).
parameter(kdmc,0.01).   parameter(ksC,1.6).     parameter(V1B,0.5).
parameter(vsB,1).       parameter(V2C,0.1).     parameter(k5,0.4).
parameter(KIB,2.2).     parameter(V1C,0.6).     parameter(vdBC,0.5).
parameter(vmB,0.8).     parameter(kdnc,0.12).   parameter(V4B,0.2).
parameter(KmB,0.4).     parameter(vdCC,0.7).    parameter(V3B,0.5).
parameter(kdmb,0.01).   parameter(V2PC,0.1).    parameter(vdBN,0.6).
parameter(n,4).         parameter(k2,0.2).      parameter(vdIN,0.8).
parameter(m,2).         parameter(V1PC,0.4).
parameter(Kd,0.3).      parameter(k1,0.4).

% mRNA
vsP*[BN]^n/(KAP^n+[BN]^n)        for _=[BN]=>MP.
vmP*[MP]/(KmP+[MP])+kdmp*[MP]    for MP=>_.

vsC*[BN]^n/(KAC^n+[BN]^n)        for _=[BN]=>MC.
vmC*[MC]/(KmC+[MC])+kdmc*[MC]    for MC=>_.

vsB*KIB^m/(KIB^m+[BN]^m)         for _=>MB.
vmB*[MB]/(KmB+[MB])+kdmb*[MB]    for MB=>_.

ksP*[MP]                         for _=[MP]=>PC.
V2P*[PCP]/(Kdp+[PCP])            for PCP=>PC.
k4*[PCC]                         for PCC=>PC+CC.
V1P*[PC]/(Kp+[PC])               for PC=>PCP.
kdn*[PC]                         for PC=>_.
k3*[PC]*[CC]                     for PC+CC=>PCC.
kdn*[PCP]+vdPC*[PCP]/(Kd+[PCP])  for PCP=>_.

ksC*[MC]                         for _=[MC]=>CC.
V2C*[CCP]/(Kdp+[CCP])            for CCP=>CC.
V1C*[CC]/(Kp+[CC])               for CC=>CCP.
kdnc*[CC]                        for CC=>_.
vdCC*[CCP]/(Kd+[CCP])+kdn*[CCP]  for CCP=>_.

V2PC*[PCCP]/(Kdp+[PCCP])         for PCCP=>PCC.
```

```
V1PC*[PCC]/(Kp+[PCC])            for PCC=>PCCP.
k2*[PCN]                         for PCN=>PCC.
k1*[PCC]                         for PCC=>PCN.
kdn*[PCC]                        for PCC=>_.

V4PC*[PCNP]/(Kdp+[PCNP])         for PCNP=>PCN.
V3PC*[PCN]/(Kp+[PCN])            for PCN=>PCNP.
k8*[In]                          for In=>BN+PCN.
k7*[BN]*[PCN]                    for BN+PCN=>In.
kdn*[PCN]                        for PCN=>_.

vdPCC*[PCCP]/(Kd+[PCCP])+kdn*[PCCP]    for PCCP=>_.

vdPCN*[PCNP]/(Kd+[PCNP])+kdn*[PCNP]    for PCNP=>_.

ksB*[MB]                         for _=[MB]=>BC.
V2B*[BCP]/(Kdp+[BCP])            for BCP=>BC.
V1B*[BC]/(Kp+[BC])               for BC=>BCP.
k6*[BN]                          for BN=>BC.
k5*[BC]                          for BC=>BN.
kdn*[BC]                         for BC=>_.

vdBC*[BCP]/(Kd+[BCP])+kdn*[BCP]  for BCP=>_.

V4B*[BNP]/(Kdp+[BNP])            for BNP=>BN.
V3B*[BN]/(Kp+[BN])               for BN=>BNP.
kdn*[BN]                         for BN=>_.

vdBN*[BNP]/(Kd+[BNP])+kdn*[BNP]  for BNP=>_.

vdIN*[In]/(Kd+[In])+kdn*[In]     for In=>_.

% Initial state

present(preMPF,0.279939269).     present(PC,0.841323888).
present(MPF,0.747404087).        present(PCP,0.117599215).
present(C25,0.629659946).        present(CC,8.03578267).
present(C25P,0.370340054).       present(CCP,0.715418129).
present(Wee1,0.00223979517).     present(PCC,5.4251231).
present(Wee1P,0.132059534).      present(PCN,3.50345817).
present(APC,0.0303504407).       present(PCCP,0.250178481).
present(X,0.41856651).           present(PCNP,0.239897171).
present(Wee1m,0.141190132).      present(BC,1.71314172).
present(APCi,0.973282491).       present(BCP,0.91889792).
present(MP,2.80754078).          present(BN,0.308181747).
present(MC,1.5903695).           present(BNP,0.243137076).
```

```
present(MB,7.85510842).          present(In,0.637094766).
present(MR,4.77799325).
```

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Unité de recherche INRIA Rocquencourt
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Unité de recherche INRIA Futurs : Parc Club Orsay Université - ZAC des Vignes
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