Modélisation mathématique de la prolifération cellulaire et de son contrôle circadien : défis en chronothérapeutique des cancers

Giving medical sense to mathematical modelling of cell proliferation and its control: challenges from cancer therapeutics

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Séminaire de mathématiques appliquées, Chaire EDP et applications, 21 mai 2010

Outline

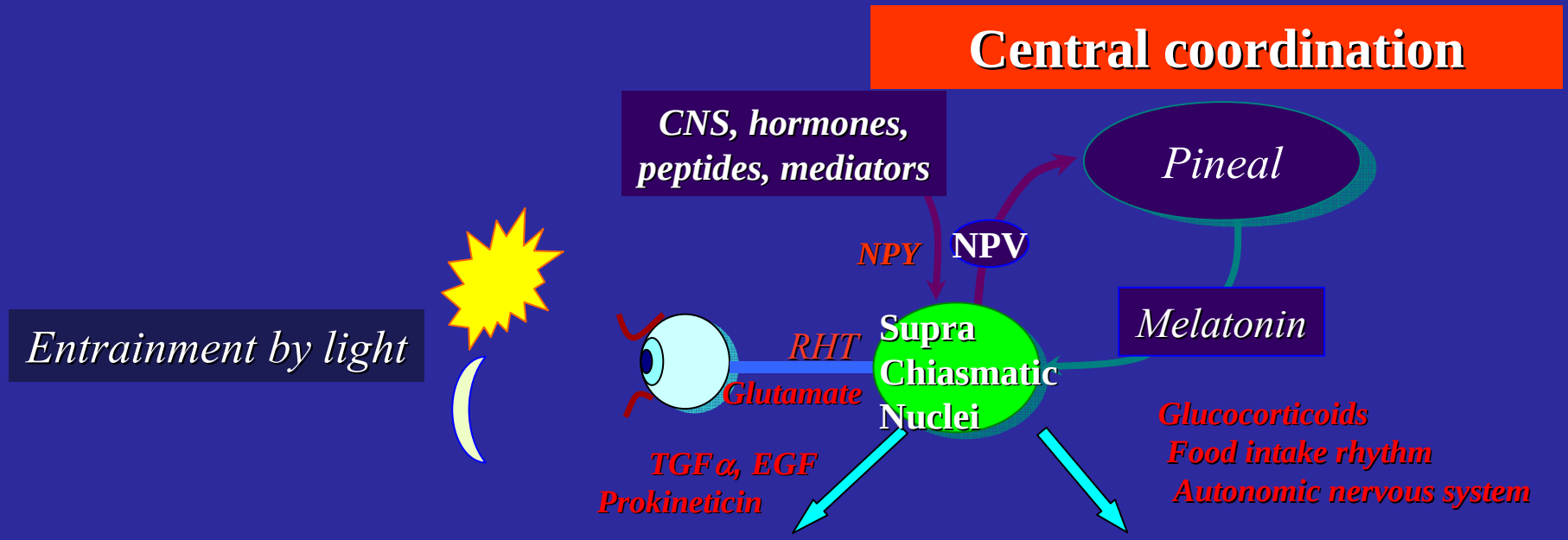
- Questions from cancer growth and cancer therapeutics
- Modelling the cell division cycle at the cell population level
- Circadian rhythm and tumour growth: possible explanations
- Modelling cell proliferation and quiescence
- Modelling molecular pharmacokinetics-pharmacodynamics (PK-PD)
- Main challenge: optimisation of cancer therapeutics
- More challenges and future prospects

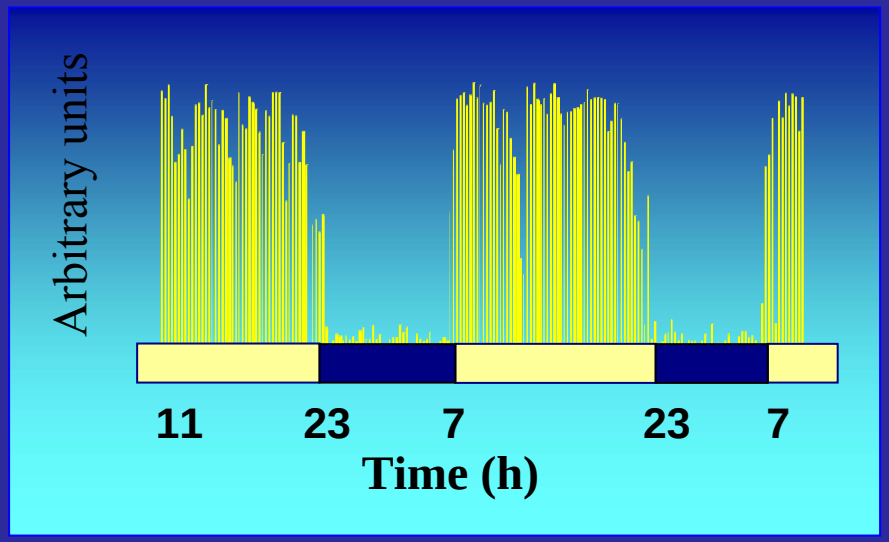
Questions from tumour growth, circadian clocks and cancer therapeutics

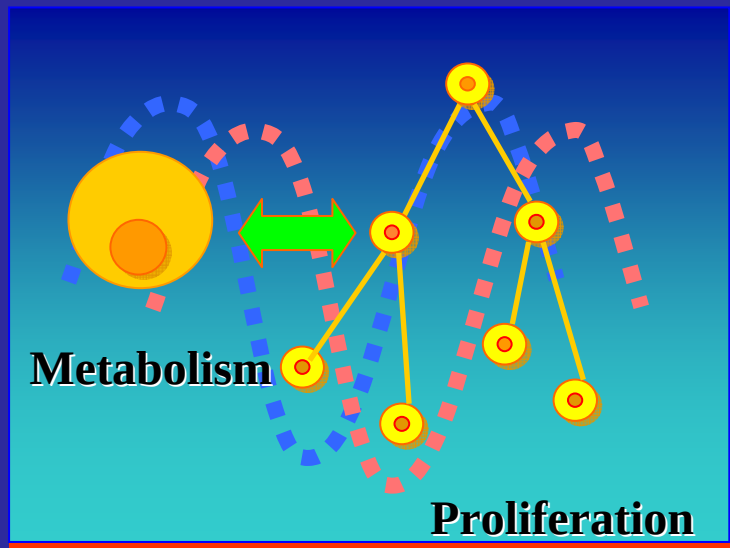
("circa diem"= about 24 h)

The circadian system…

Central coordination







Rest-activity cycle: open window on SCN central clock

Peripheral oscillators

Lévi, Lancet Oncol 2001 ; Mormont & Lévi, Cancer 2003

(from Francis Lévi, INSERM U 776 Rythmes Biologiques et Cancers)

…is an orchestra of cell clocks with one neuronal conductor in the SCN and molecular circadian clocks in all nucleated cells

SCN=suprachiasmatic nuclei (in the hypothalamus)

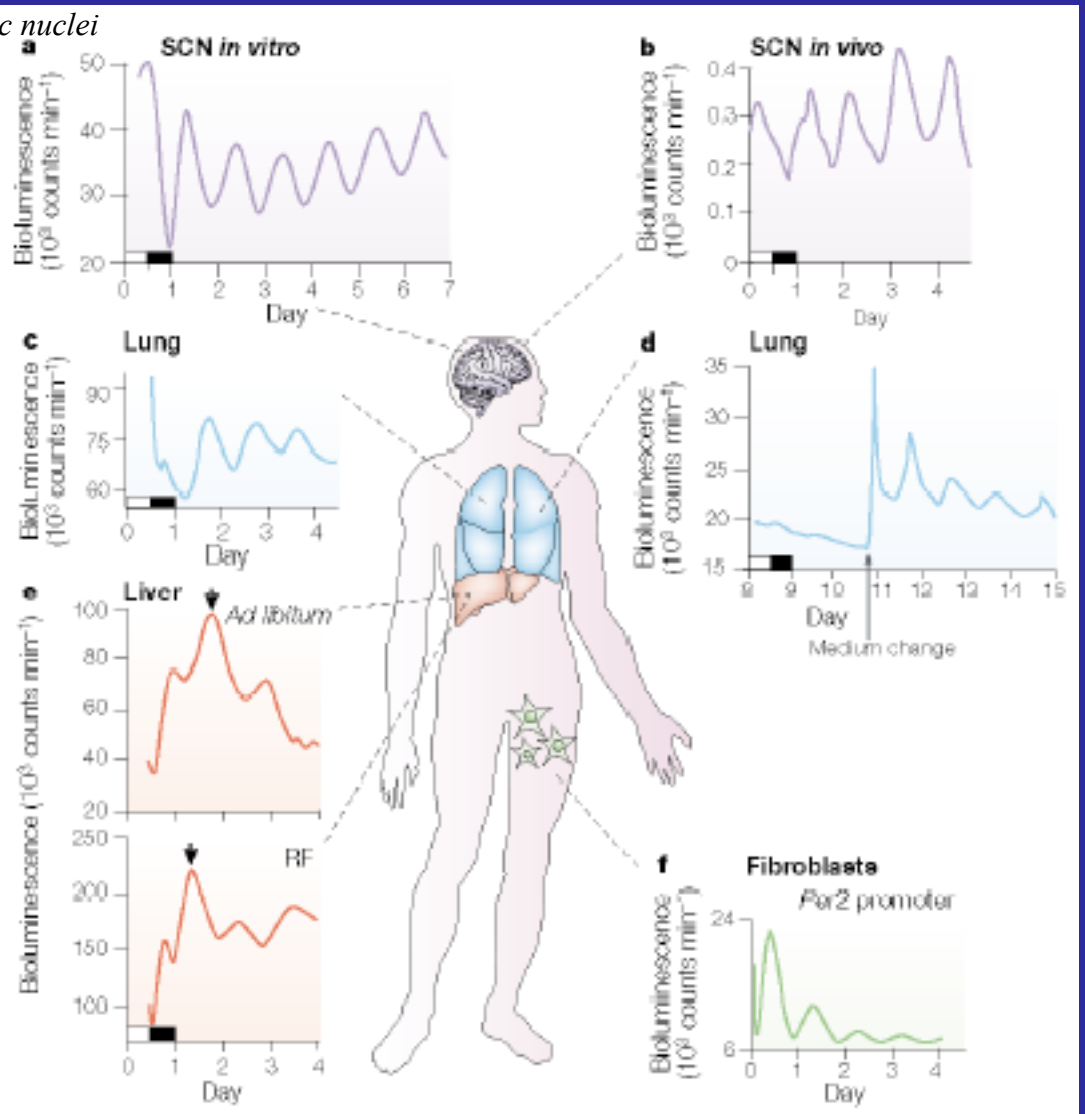
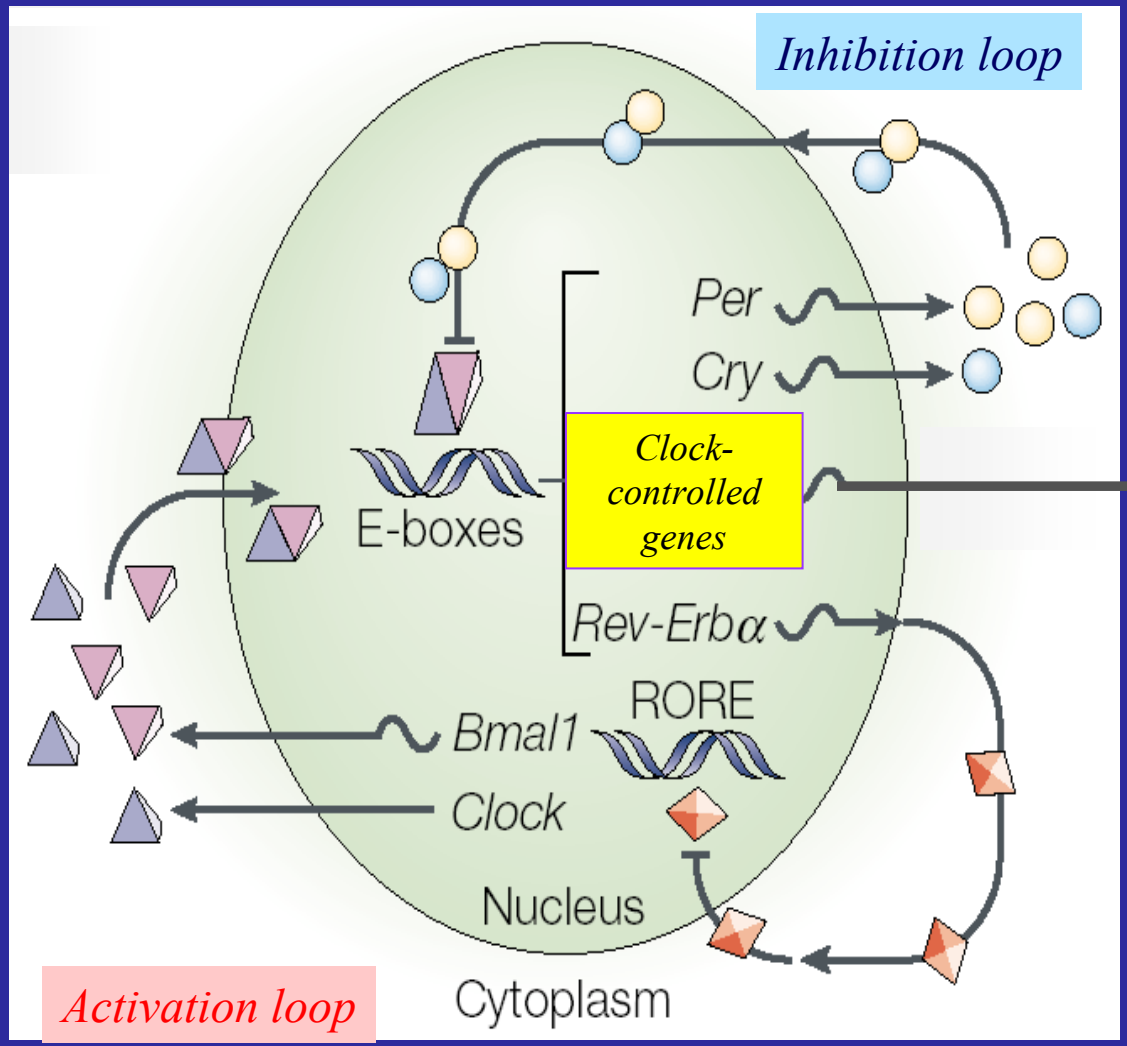


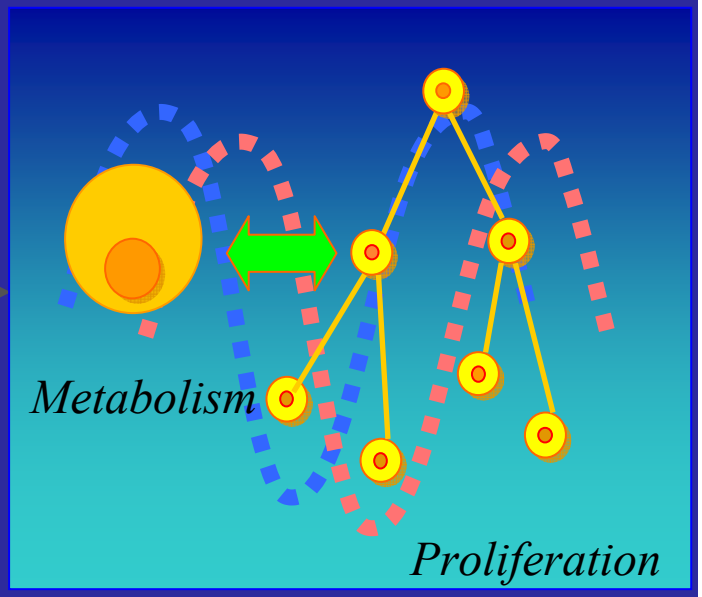
Figure 3 | ***Per::luciferase* transgenes reveal a diversity of tissue-based circadian oscillators.**

(from Hastings, Nature Rev.Neurosci. 2003)

In each nucleated cell: a molecular circadian clock



Cellular rhythms

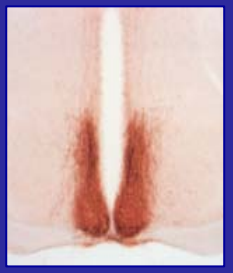


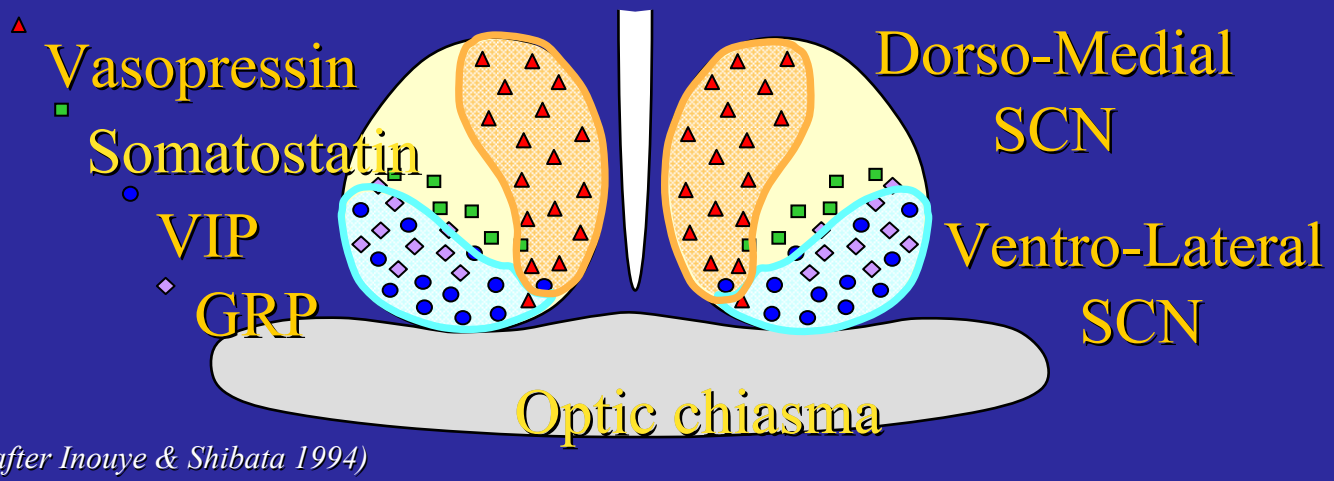
24 h-rhythmic transcription:
10% of genome, among which:
10% : cell cycle
2% : growth factors

(after Hastings, Nature Rev. Neurosci. 2003)

(from Francis Lévi, INSERM U 776 Rythmes Biologiques et Cancers)

The central circadian pacemaker: the suprachiasmatic (SCN) nuclei





(after Inouye & Shibata 1994)

20 000 coupled neurons, in particular electrically (coupling blocked by TTX), each one of them oscillating according to a period ranging between 20 et 28 h

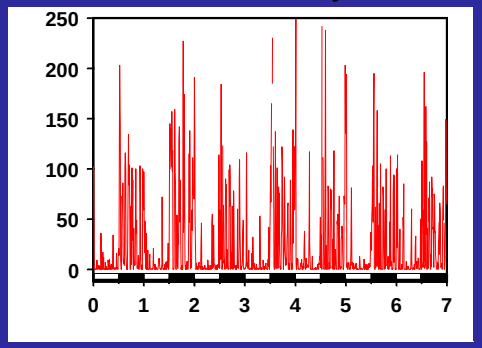
With entrainment by light (through the retinohypothalamic tract) for VL neurons

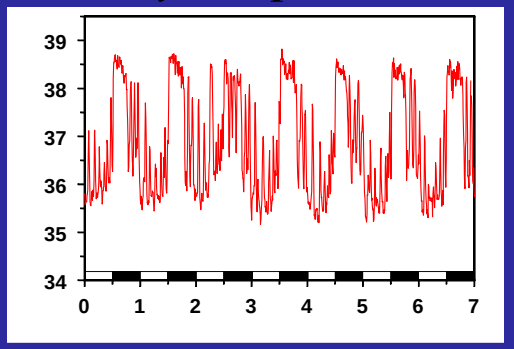
Circadian rhythm disruption by SCN perturbations in mice

Rest-activity

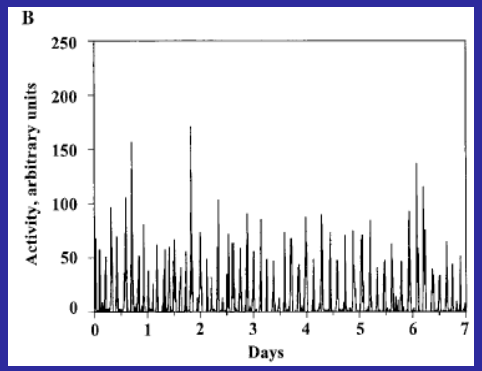
Body temperature

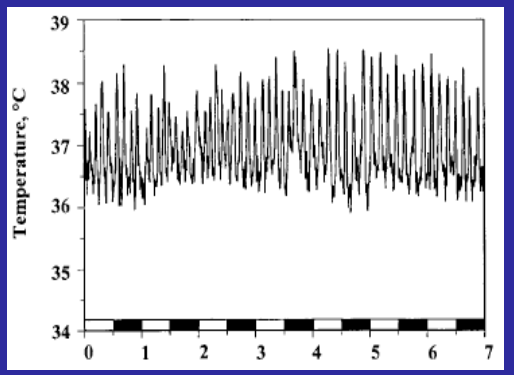
Intact SCN



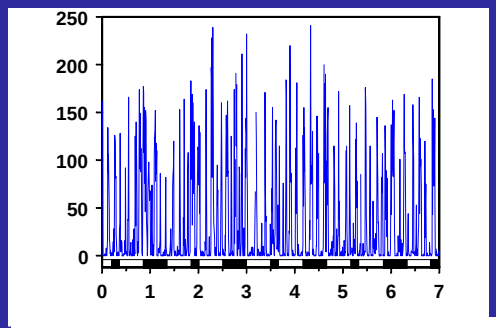


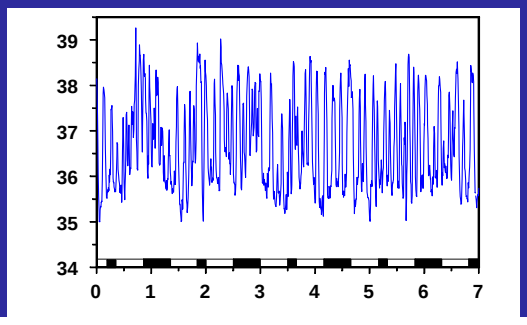
Electrocoagulation





Intact+Jet-lag





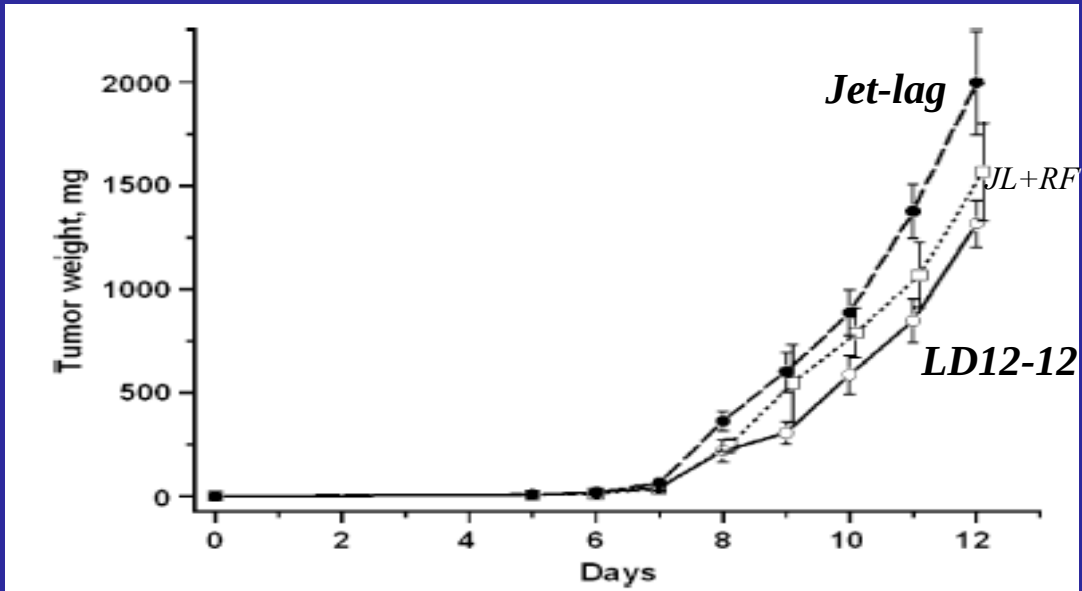
Filipski JNCI 2002, Canc. Res. 2004, JNCI 2005, Canc. Causes Control 2006

A question from animal physiopathology: tumour growth and circadian clock disruption

Observation: a circadian rhythm perturbation by chronic jet-lag-like light entrainment (phase advance) enhances GOS tumour proliferation in $B6D2F_1$ mice

LD12-12

Jet-lag



Filipski JNCI 2002, Canc. Res. 2004, JNCI 2005, Canc. Causes Control 2006

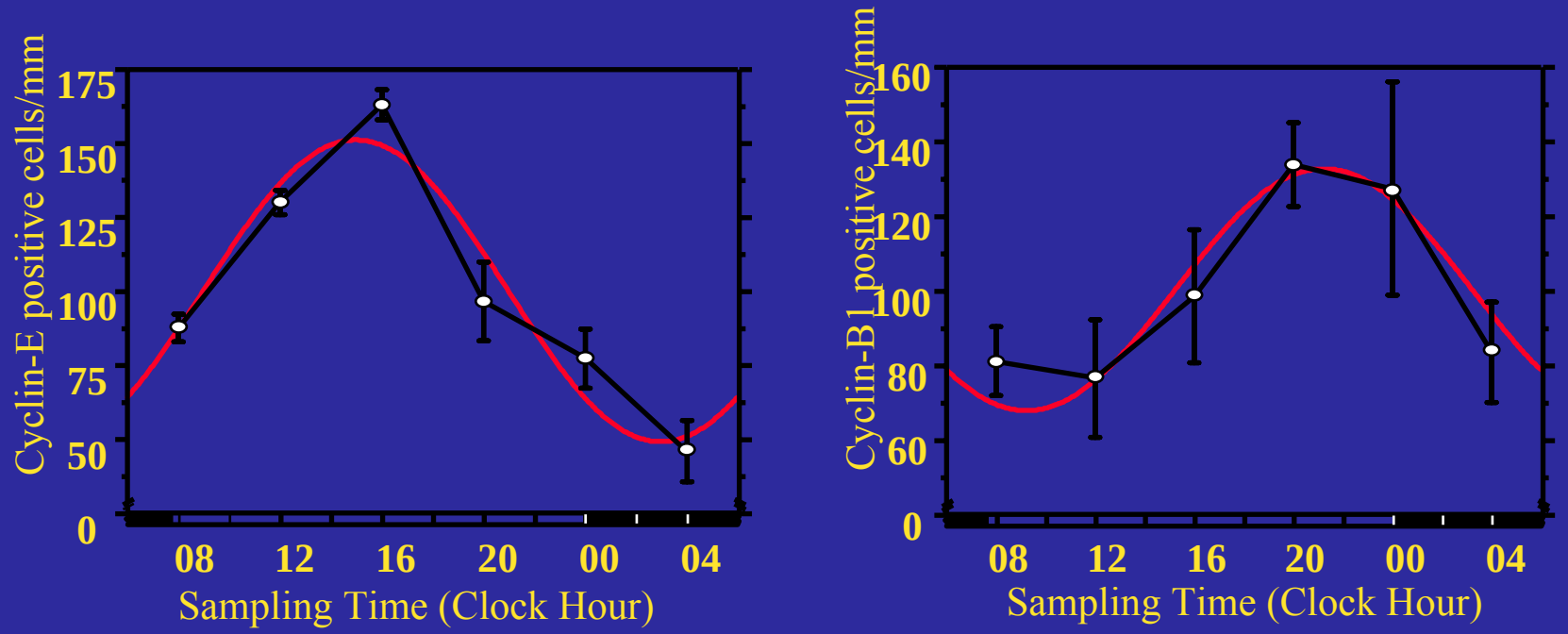
Here, clearly:
λ(Jet-lag) > λ(LD 12-12)
if λ is a growth exponent

How can this be accounted for in a mathematical model of tumour growth?
Major public health stake! (does shift work enhance the incidence of cancer in Man?)

(The answer is yes, cf. e.g. Davis, S., Cancer Causes Control 2006)

Human physiology: circadian rhythms in the Human cell division cycle

Example of circadian rhythm in normal (=homeostatic) Human oral mucosa for Cyclin E (control of G_1/S transition) and Cyclin B (control of G_2/M transition)



Nuclear staining for Cyclin-E and Cyclin-B1. Percentages of mean ± S.E.M. in oral mucosa samples from 6 male volunteers. Cosinor fitting, $p < 0.001$ and $p = 0.016$, respectively.

(after Bjarnason et al. Am J Pathol 1999)

Circadian rhythm disruption in Man

[= loss of synchronisation between circadian molecular clocks?]

- Circadian desynchronisation (loss of rythms of temperature, cortisol, rest-activity) is a factor of poor prognosis in response to anticancer chemotherapy *(Mormont & Lévi, Cancer 2003)*

- Desynchronising effects of *cytokines* and anticancer drugs on circadian clock: *fatigue* is a constant symptom in patients with cancer *(Rich et al., Clin Canc Res 2005)*

- …effects that are analogous to those of chronic « jet-lag » (photic entrainment phase advance or delay) on circadian rhythms, known to enhance tumour growth
  (Hansen, Epidemiol 2001; Schernhammer, JNCI 2003; Davis, JNCI 2001, Canc Causes Control 2006)

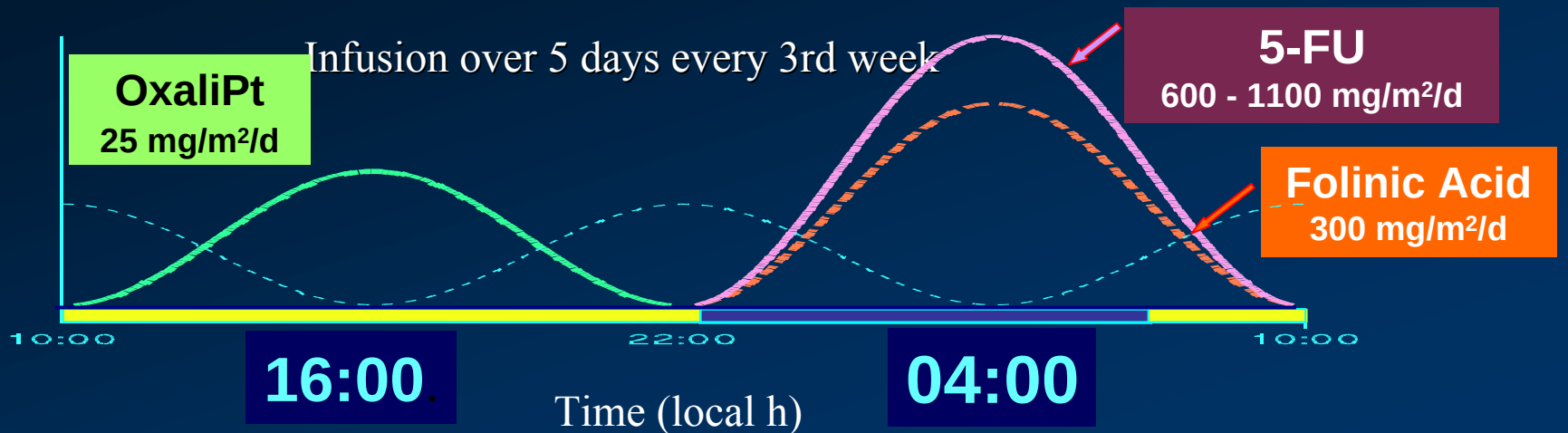
- *…hence questions: 1) is the molecular circadian clock the main synchroniser between phase transitions? 2) do tumours enhance their development by disrupting the SCN clock?*

- […and hence resynchronisation therapies (by melatonin, cortisol) in oncology??]

Circadian rhythms and cancer chronotherapeutics

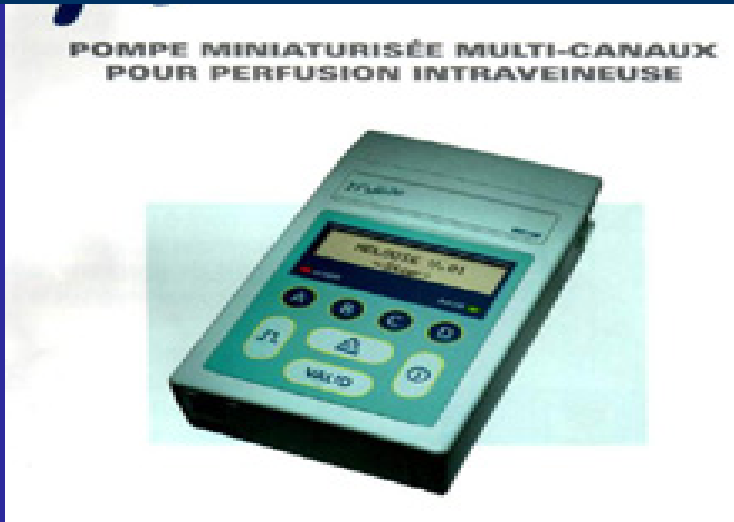
(Results from Francis Lévi's INSERM team U 776, Villejuif, France)

Time-scheduled delivery regimen for metastatic CRC



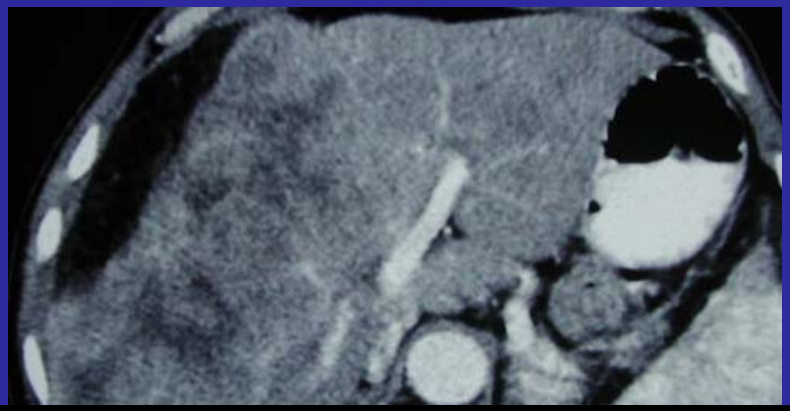
Multichannel programmable ambulatory injector for intravenous drug infusion (pompe Mélodie, Aguettant, Lyon, France)

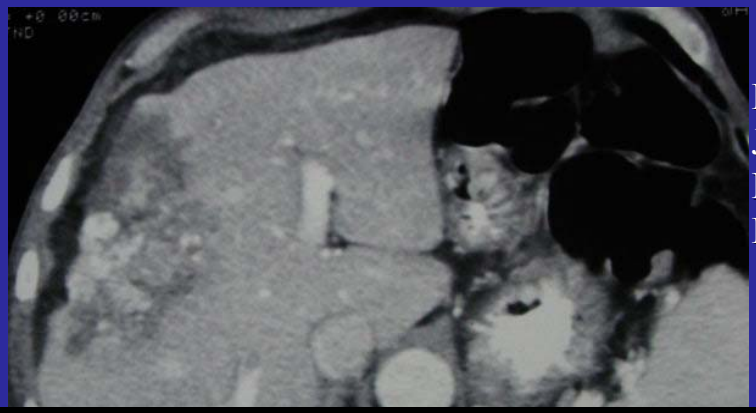
Can such therapeutic schedules be improved?



Results of cancer chronotherapy

Metastatic colorectal cancer (Folinic Acid, 5-FU, Oxaliplatin)	Infusion flow		
	Constant	Chrono	
Toxicity			p
Oral mucositis gr 3-4	74%	14%	$<10^{-4}$
Neuropathy gr 2-3	31%	16%	$<10^{-2}$
Responding rate	30%	51%	$<10^{-3}$





Lévi et al.
JNCI 1994 ;
Lancet 1997 ;
Lancet Oncol 2001

Possible explanations: impact of circadian clocks on both cell drug detoxication enzymes and cell division cycle determinant proteins

Modelling cell proliferation at the cell population level

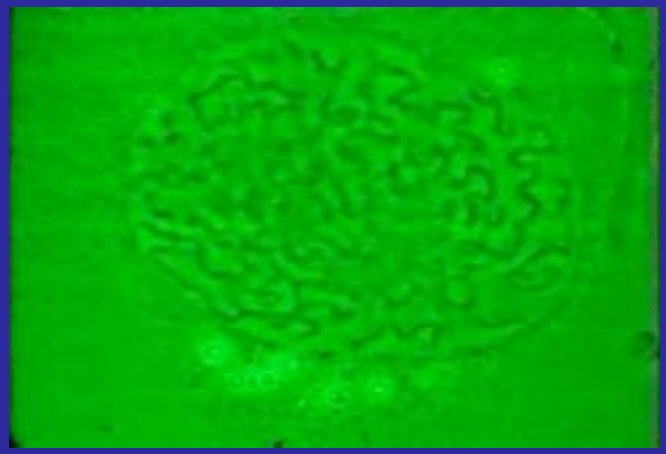
Cell population growth in proliferating tissues

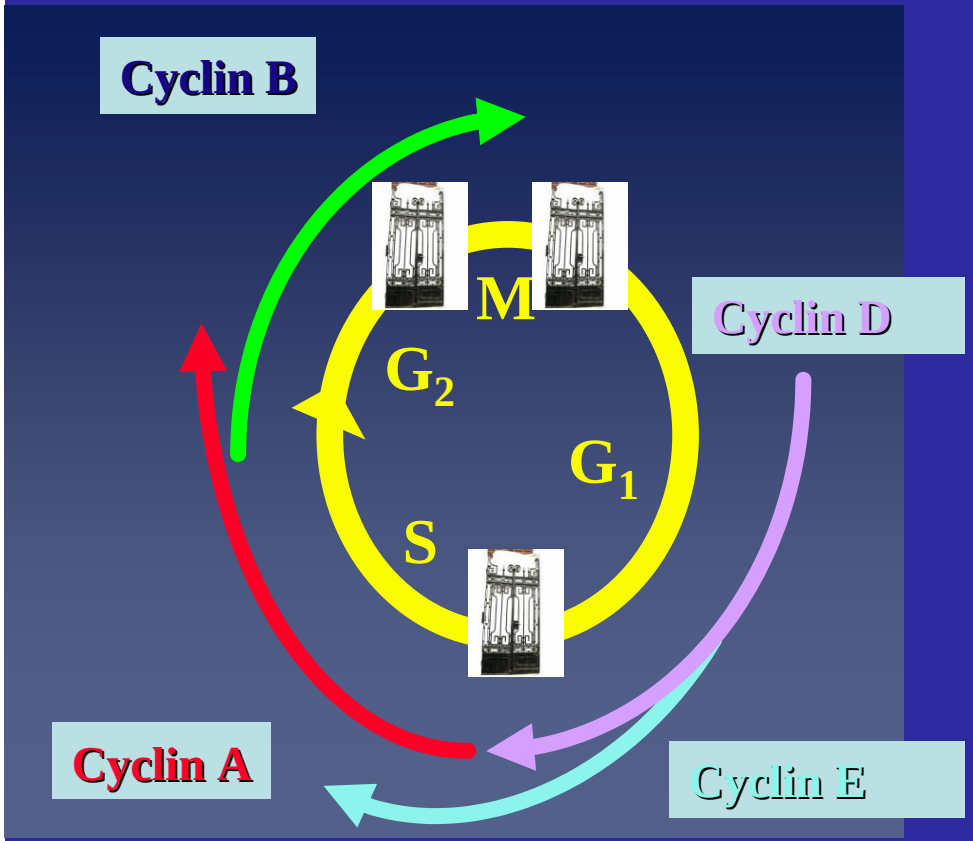
(from Lodish et al., Molecular cell biology, Nov. 2003)

One cell divides in two: a physiologically controlled process at cell and tissue levels in all fast renewing tissues (gut, skin, bone marrow…) that is *disrupted in cancer*

At the origin of proliferation: the cell division cycle

S:=DNA synthesis; G_1,G_2:=Gap1,2; M:=mitosis ►





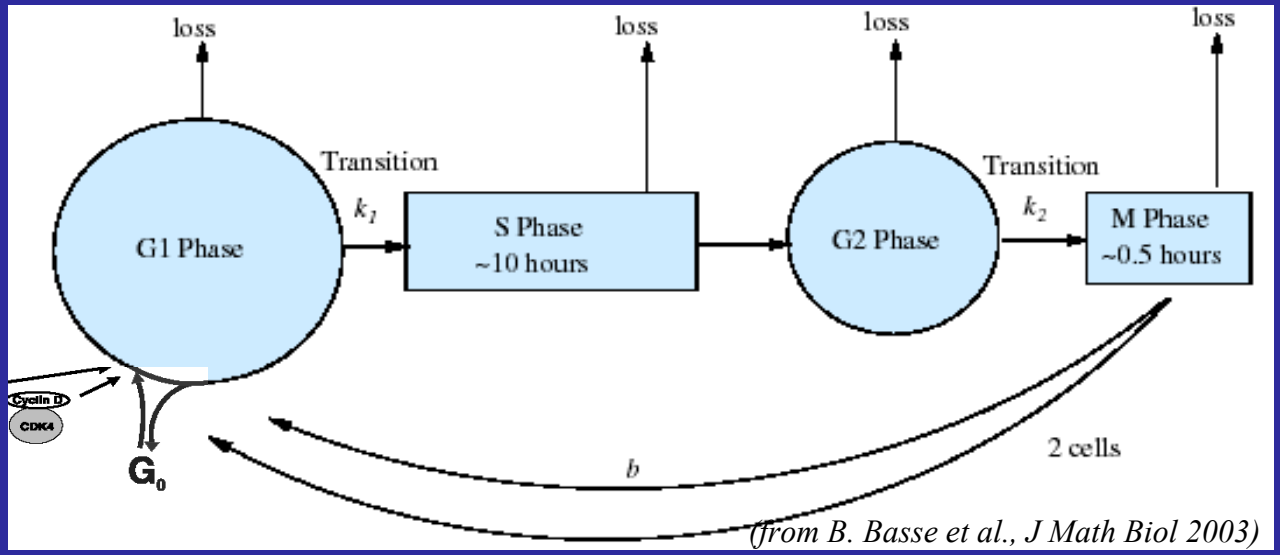
(from Lodish et al., Molecular cell biology, 2003)

Physiological or therapeutic control exerted on:

- transitions (checkpoints) between phases (G_1/S, G_2/M, M/G_1)
- death rates (apoptosis or necrosis)
- progression speeds inside phases
- exchanges between quiescent (G_0) and proliferative phases (G_1 only)

Modelling the cell division cycle in cell populations

Age-structured PDE models



(from B. Basse et al., J Math Biol 2003)

In each phase *i*, a Von Foerster-McKendrick-like equation:

$$\frac{\partial}{\partial t} n_i(t,a) + \frac{\partial}{\partial a}[v_i(a) n_i(t,a)] + d_i(t,a) n_i(t,a) + K_{i\to i+1}(t,a) n_i(t,a) = 0$$

$$v_i(0) n_i(t, a=0) = \int_{\alpha \geq 0} K_{i-1\to i}(t,\alpha)\, n_{i-1}(t,\alpha)\, d\alpha$$

$$K_{i\to i+1}(t,a) = \psi(t) \mathbf{1}_{a \geq a_i}(a)$$

n_i:=cell population density in phase i;
v_i:=progression speed;
d_i:=death rate;

$K_{i-1->i}$:=transition rate (with a factor 2 if $i=1$)

d_i, $K_{i->i+1}$ constant or periodic w. r. to time t ($1 \leq i \leq I$, $I+1=1$)

Death rates d_i: ("loss"), "speeds" v_i and phase transitions $K_{i->i+1}$ are model targets for physiological (e.g. circadian) and therapeutic (drugs) control $\psi(t)$

[$\psi(t)$: e.g., clock-controlled Cdk1 or intracellular output of drug infusion flow]

(Firstly presented in: JC, B. Laroche, S. Mischler, B. Perthame, RR INRIA #4892, 2003)

The simplest case: 1-phase model with division

$$\frac{\partial}{\partial t}n(t,a)+\frac{\partial}{\partial a}[n(t,a)]+[d(t)+K(t,a)]\,n(t,a)=0$$

$$n(t,a=0)=2\int_{\alpha\geq 0}K(t,\alpha)\,n(t,\alpha)\,d\alpha$$

$$\text{where } K(t,a)=K_0\psi(t)\mathbb{1}_{[a^*,+\infty[}(a)$$

$$\text{and } \psi(t)=\mathbb{1}_{[0,\tau[}(t),\ 1\text{-periodic}$$

(Here, $v(a)=1$, a^* is the total cell cycle duration, and $\tau(<1)$ is the time during which the 1-*periodic control* ψ is actually exerted on cell division)

Then it can be shown that the eigenvalue problem: $n(t,a)=e^{\lambda t}N(t,a)$

$$\frac{\partial}{\partial t}N(t,a)+\frac{\partial}{\partial a}[N(t,a)]+[\lambda+d(t)+K(t,a)]\,N(t,a)=0$$

$$N(t,a=0)=2\int_{\alpha\geq 0}K(t,\alpha)\,N(t,\alpha)\,d\alpha$$

has a unique positive 1-*periodic* eigenvector N, with a positive eigenvalue λ and an explicitformula can be found for λ when $K_0 \longrightarrow +\infty$ *(Th. Lepoutre's PhD thesis)*

General case (N phases): by the Krein-Rutman theorem (infinite-dimensional form of the Perron-Frobenius theorem), there exists a nonnegative first eigenvalue λ and, if $\widetilde{N}_i(t,a) = e^{-\lambda t} n_i(t,a)$, N_i, bounded solutions to the problem *(here $v_i(a)=1$)* :

$$\begin{cases} \frac{\partial}{\partial t} N_i(t,a) + \frac{\partial}{\partial a} N_i(t,a) + [d_i(t,a) + \lambda + K_{i\to i+1}(t,a)] N_i(t,a) = 0, \\ N_i(t,a=0) = \int_{\alpha\geq 0} K_{i-1\to i}(t,\alpha)\, N_{i-1}(t,\alpha)\, d\alpha, \quad 2 \leq i \leq I \\ N_1(t,a=0) = 2\int_{\alpha\geq 0} K_{I\to 1}(t,\alpha)\, N_I(t,\alpha)\, d\alpha, \quad \text{with} \sum_{i=1}^{I} \int_{a\geq 0} N_i(t,a)da = 1 \end{cases}$$

with a number ρ such that the asymptotics of $\widetilde{N}_i(t,a) = e^{-\lambda t} n_i(t,a)$ follow:

$$\int_{\alpha\geq 0} \left| \widetilde{N}_i(t,\alpha) - \rho.\, N_i(t,\alpha) \right| \varphi_i(t,\alpha) d\alpha \to 0 \quad \text{as} \quad t \to \infty$$

(the weights φ_i being solutions to the dual problem); this can be shown by using a generalised entropy principle (GRE). Moreover, if the control (on d_i or $K_{i->i+1}$) is constant, or if it is periodic, so are the N_i, with the same period in the periodic case.

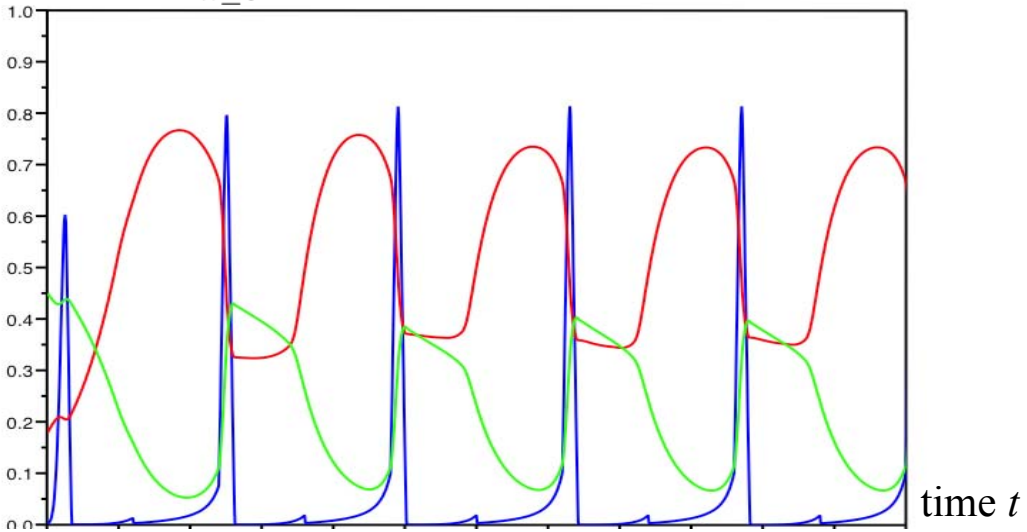
Ph. Michel, S. Mischler, B. Perthame, C. R. Acad. Sci. Paris Ser. I (Math.) 2004; J Math Pures Appl 2005
JC, Ph. Michel, B. Perthame, C. R. Acad. Sci. Paris Ser. I (Math.) 2006; Proc. ECMTB Dresden 2005, Birkhäuser 2007

λ: a growth exponent governing the cell population behaviour

Proof of the existence of *a unique growth exponent* λ, the same for all phases *i*, such that the $\widetilde{N}_i(t,a) = e^{-\lambda t} n_i(t,a)$ are bounded and asymptotically periodic if the control is periodic

Surfing on the exponential growth curve, example (periodic control case): 2 phases, control on G_2/M transition by 24-h-periodic CDK1-Cyclin B (A. Goldbeter's model)

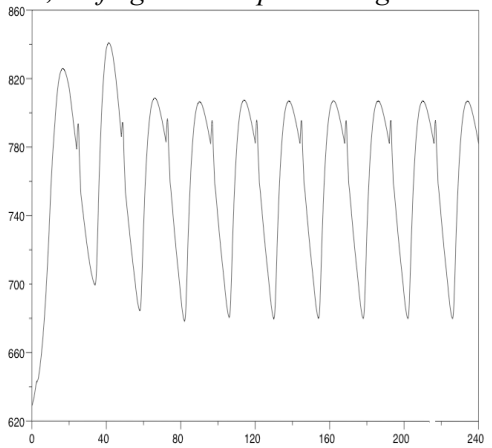
$$\mathcal{N}_i^{tot}(t) = e^{-\lambda t} \int_{\alpha \geq 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$ (Normalised cell population number)



ψ=CDK1 **All cells in G1-S-G2 (phase *i*=1)** **All cells in M (phase *i*=2)**

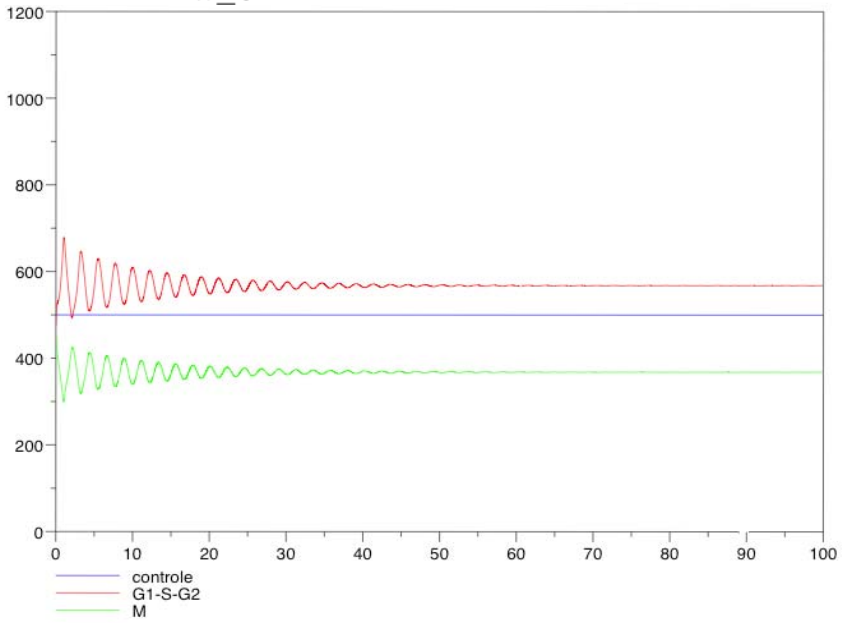
Entrainment of the cell division cycle by CDK1 at the circadian period

All cells, surfing on the exponential growth curve



Details (1): 2 phases, no control on G_2/M transition

$$\mathcal{N}_i^{tot}(t) = e^{-\lambda t} \int_{\alpha \geq 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$

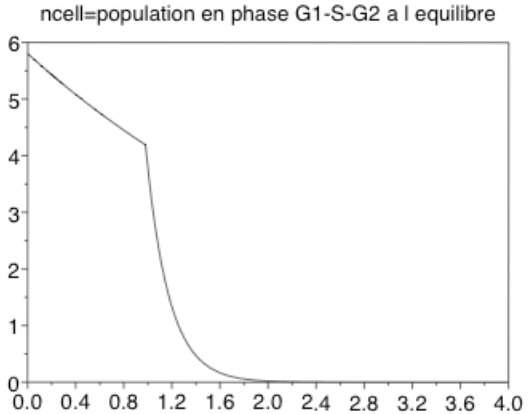


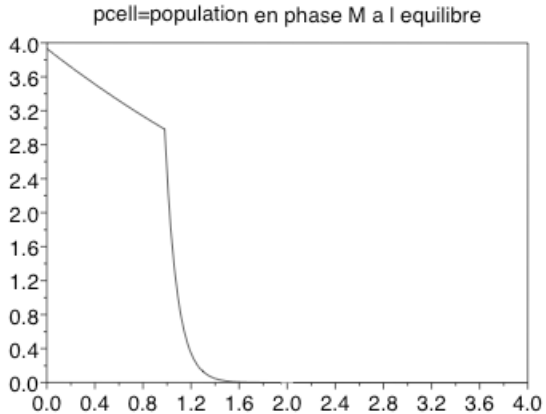
The total population of cells

$$\int_{\alpha > 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$

inside each phase follows asymptotically an exponential behaviour

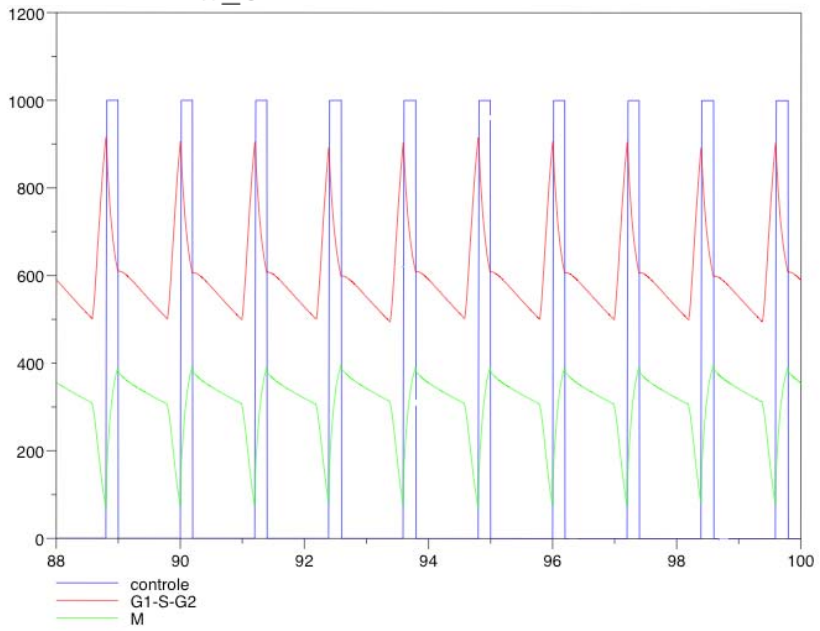
Stationary state distribution of cells inside phases according to age *a*: *no control, hence exponential decay*





Details (2): 2 phases, periodic control ψ on G_2/M transition

$$\mathcal{N}_i^{tot}(t) = e^{-\lambda t} \int_{\alpha \geq 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$

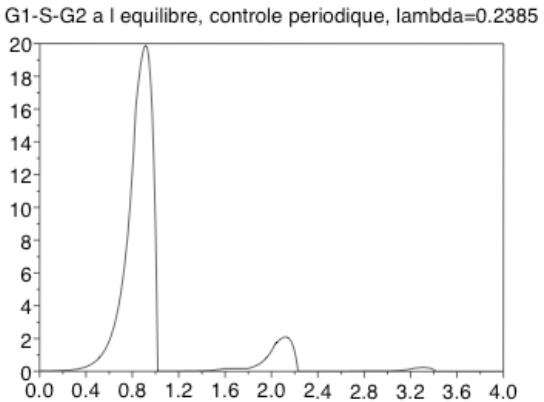


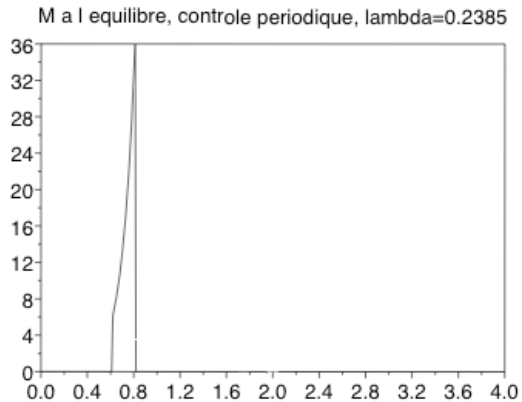
The total population of cells

$$\int_{\alpha > 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$

inside each phase follows asymptotically an exponential behaviour *tuned by a periodic function*

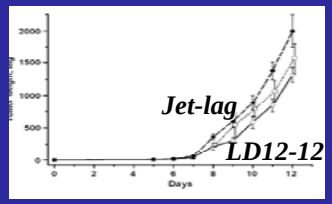
Stationary state distribution of cells inside phases according to age *a: sharp periodic control, hence sharp rise and decay*





Circadian rhythm and tumour growth: searching for possible explanations

Circadian rhythm and tumour growth: How can we define and compare the λs?



λ(Jet-lag) > λ(LD 12-12)

Instead of the initial system with periodic coefficients:

$$\begin{cases} \frac{\partial}{\partial t}N_i(t,a) + \frac{\partial}{\partial a}N_i(t,a) + [d_i(t,a) + \lambda + K_{i\to i+1}(t,a)]N_i(t,a) = 0, \\ N_i(t,a=0) = \int_{\alpha\geq 0} K_{i-1\to i}(t,\alpha)\, N_{i-1}(t,\alpha)\, d\alpha, \quad 2 \leq i \leq I \\ N_1(t,a=0) = 2\int_{\alpha\geq 0} K_{I\to 1}(t,\alpha)\, N_I(t,\alpha)\, d\alpha, \quad \text{with} \sum_{i=1}^{I}\int_{a\geq 0} N_i(t,a)da = 1 \end{cases} \rightarrow \lambda_{per}$$

Define the stationary system with constant coefficients:

$$\begin{cases} \frac{\partial}{\partial x}\bar{N}_i(x) + [\langle d_i(x)\rangle_a + \lambda_{stat} + \langle K_{i\to i+1}(x)\rangle_a]\bar{N}_i(x) = 0, \\ \bar{N}_i(x=0) = \int_{\xi\geq 0} \langle K_{i-1\to i}(\xi)\rangle_a\, \bar{N}_{i-1}(\xi)\, d\xi, \quad 2 \leq i \leq I \\ \bar{N}_1(x=0) = 2\int_{\xi\geq 0} \langle {}_aK_{I\to 1}(\xi)\rangle_a\, \bar{N}_I(\xi)\, d\xi, \quad \text{with} \sum_{i=1}^{I}\int_{x\geq 0} \bar{N}_i(x)dx = 1 \end{cases} \rightarrow \lambda_{stat}$$

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

(JC, Ph. Michel, B. Perthame, C. R. Acad. Sci. Paris Ser. I (Math.) 2006; Proc. ECMTB Dresden 2005, Birkhäuser 2007)

Comparing λ_{per} and λ_{stat}: control on apoptosis only

(comparison of periodic versus constant [=no] control with same mean)

Theorem (B. Perthame, 2005):

If the control is exerted on the sole loss (apoptosis) terms d_i, then $\lambda_{per} \geq \lambda_{stat}$

i.e., λ(periodic control) $\geq \lambda$(constant control)

if the control is on the d_i only

… which is exactly the contrary of what was expected, at least if one assumes that

$\lambda_{per} \approx \lambda$*(LD12-12)* and $\lambda_{stat} \approx \lambda$*(jet-lag) !*

(JC, Ph. Michel, B. Perthame, C. R. Acad. Sci. Paris Ser I (Math), 2006; Proc. ECMTB Dresden 2005, Birkhäuser 2007)

Comparing λ_{per} and λ_{stat}: control on transitions only

(comparison of periodic versus constant [=no] control with same mean)

Numerical results for the periodic control of the cell cycle on phase transitions

$$\left(K_{i->i+1}(t, a) = \psi_i(t) \, . \, 1_{\{a \geq a_i\}}(a)\right)$$

Two phases, control ψ on phase transition 1->2 only:

both situations may be observed, i.e., $\lambda_{stat} <$ or $> \lambda_{per}$
depending on the duration ratio between the two phases and on the control:

ψ_1: G2/M gate open 4 h / closed 20 h

(G1-S-G2 / M)	(periodic)	(constant)
time ratio, ψ_1	λ_{per}	λ_{stat}
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

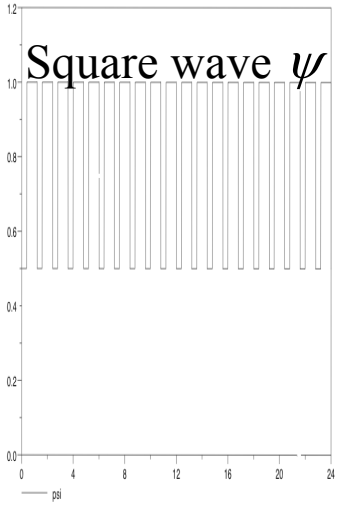
ψ_2: G2/M gate open 12 h / closed 12 h

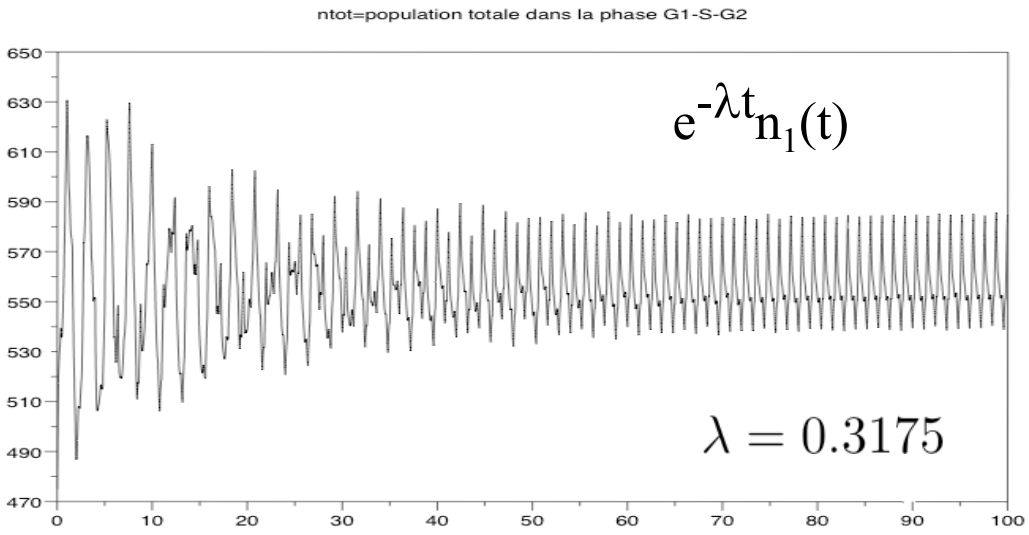
(G1-S-G2 / M)	(periodic)	(constant)
time ratio, ψ_2	λ_{per}	λ_{stat}
1	0.2623	<u>0.2821</u>
2	0.3265	<u>0.3448</u>
3	...	...
4	...	...
5	...	...
6	...	...
7	0.4500	<u>0.4529</u>
8	<u>0.4588</u>	0.4575
10	<u>0.4713</u>	0.4641
20	<u>0.5006</u>	0.4818

(JC, Ph. Michel, B. Perthame, C. R. Acad. Sci. Paris Ser I (Math.) 2006; Proceedings ECMTB Dresden 2005, Birkhäuser, 2007)

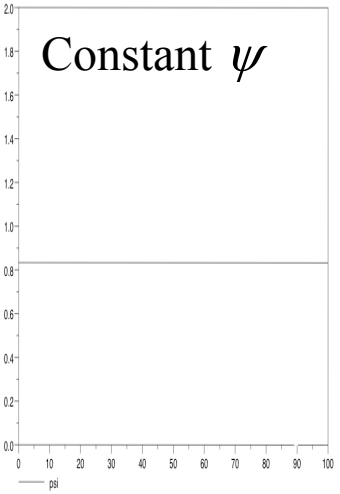
Example: ψ=1(16h)+.5(8h) sq. wave vs. constant (=no) control

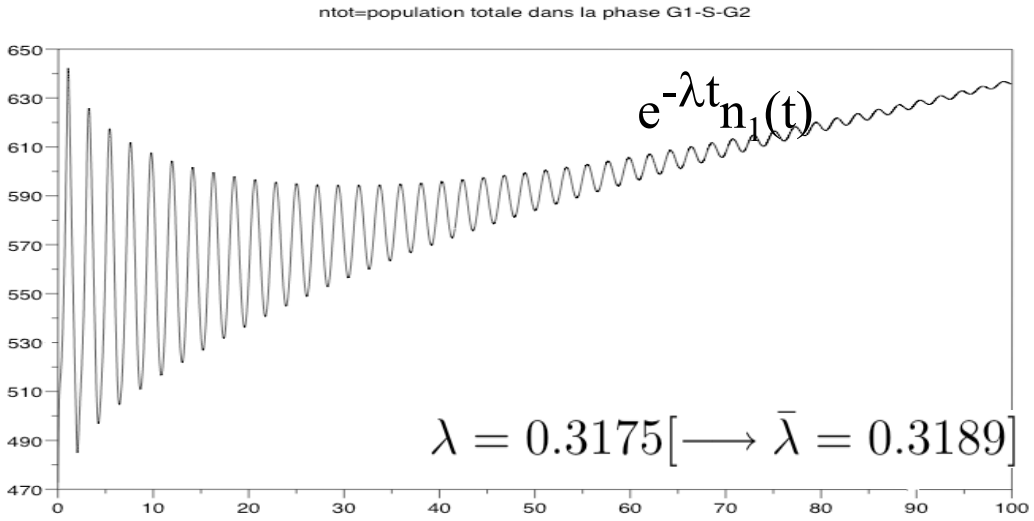
Two phases





Two phases





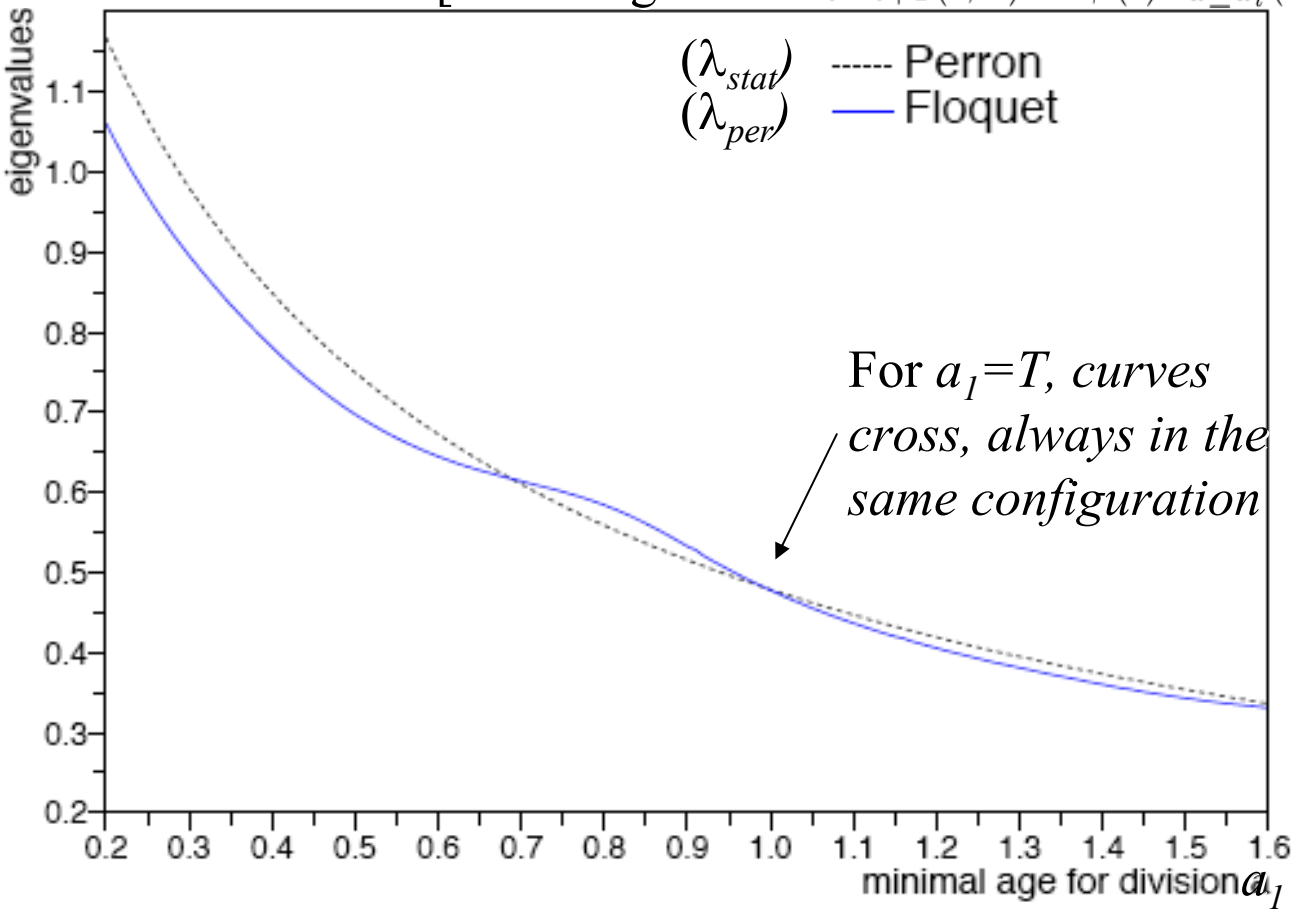
(Here: 2 cell cycle phases of equal duration, control exerted on G_2/M transition)

Theorem (Th. Lepoutre, 2008): (control on mitotic transition, $d=0$)

No hierarchy can exist in general between λ_{per} and λ_{stat},

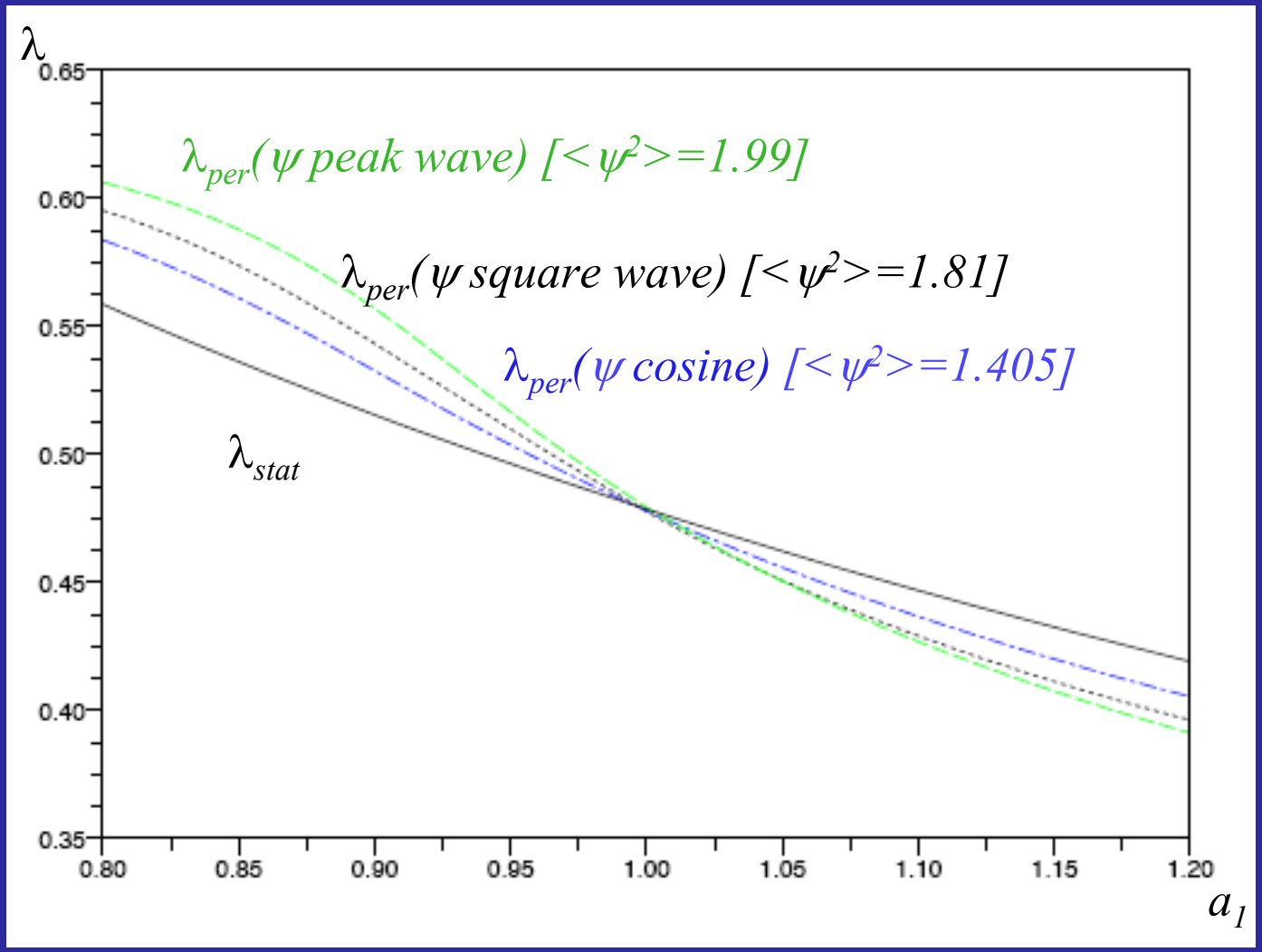
proof for a 1-phase model [illustrated here with control $\psi(\square)\square 1+0.9\cos 2\pi t/T$]

[Recalling that $K_{i\to i+1}(t,a) = \psi(t)\mathbf{1}_{a\geq a_i}(a)$]



(Th. Lepoutre's PhD thesis 2009; JC, S. Gaubert, Th. Lepoutre , MMNP 2009)

Details on crossing curves around $a_1=T$ (period of ψ) for different shapes of control ψ on mitosis



(Th. Lepoutre's PhD thesis 2009; JC, S. Gaubert, Th. Lepoutre MMNP 2009)

Nevertheless note also:

Theorem (S. Gaubert and B. Perthame, 2007):

The first result $\lambda_{per} > \lambda_{stat}$ holds for control exerted on both the d_i and the $K_{i->i+1}$ ...

...but provided that one uses for λ_{stat} *an arithmetico-geometric mean* for the $K_{i->i+1}$ *:*

$$\begin{cases} \frac{\partial}{\partial x}\bar{N}_i(x) + [\langle d_i(x)\rangle_a + \lambda_{stat} + \langle K_{i\to i+1}(t,x)\rangle_a]\bar{N}_i = 0 \ , \\ \bar{N}_i(x=0) = \int_{\xi\geq 0} \langle K_{i-1\to i}(t,\xi)\rangle_g \, \bar{N}_{i-1}(\xi)d\xi, \ i \neq 1 \ , \\ \bar{N}_1(x=0) = 2\int_{\xi\geq 0} \langle K_{I\to 1}(t,\xi)\rangle_g \, \bar{N}_I(\xi)d\xi \ . \end{cases}$$

$$\begin{cases} \langle d_i(x)\rangle_a = \frac{1}{T}\int_0^T d_i(t,x)dt, \qquad \langle K_{i\to i+1}(t,x)\rangle_a = \frac{1}{T}\int_0^T K_{i\to i+1}(t,x)dt \ , \\ \langle K_{i\to i+1}(t,x)\rangle_g = \exp\left(\frac{1}{T}\int_0^T \log\left(K_{i\to i+1}(t,x)\right)dt\right) \ . \end{cases}$$

JC, S. Gaubert, B. Perthame C. R. Acad. Sci. Ser. I (Math.) Paris, 2007;
generalised in JC, S. Gaubert, T. Lepoutre Math Computer Modelling, in revision 2010

...which so far leaves open the question of accurately representing jetlag-like perturbed control of light inputs onto circadian clocks (most likely not by suppressing it!)

A result that generalises the previous one:

Theorem (S. Gaubert and T. Lepoutre, 2009):

Using an even more general model of renewal with periodic control of birth and death rates,

$$\begin{cases} \partial_t n_i(t,x) + \partial_x n_i(t,x) + d_i(t,x) n_i(t,x) = 0, \quad 1 \leq i \leq I \\ n_i(t,0) = \sum_j \int_0^\infty B_{ij}(t,x) n_j(t,x) dx. \end{cases}$$

Then it can be shown that the dominant eigenvalue λ_F (F for Floquet) of the system is convex *with respect to death rates and* geometrically convex *with respect to birth rates, i.e.,*

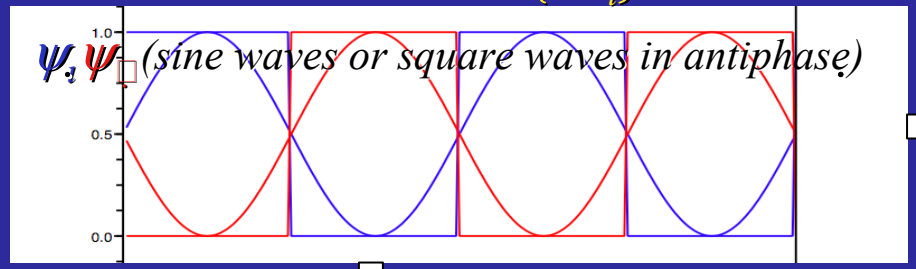
Birth rates	Death rates	Dominant eigenvalue	Inequalities
$B^1_{j\to i}$	d^1_i	λ^1_F	
$B^2_{j\to i}$	d^2_i	λ^2_F	
$(B^1_{j\to i})^\theta (B^2_{j\to i})^{1-\theta}$	$\theta d^1_i + (1-\theta) d^2_i$	λ^θ_F	$\lambda^\theta_F \leq \theta\lambda^1_F + (1-\theta)\lambda^2_F$

(using Jensen's inequality, the previous theorem results from this one)

Another possible explanation: Desynchronisation between cells with respect to cell cycle phases? *3 phase-model*: phase-opposed periodic control functions ψ_1 ($\tilde{G}_1S$) and ψ_2 ($\tilde{G}_2M$)

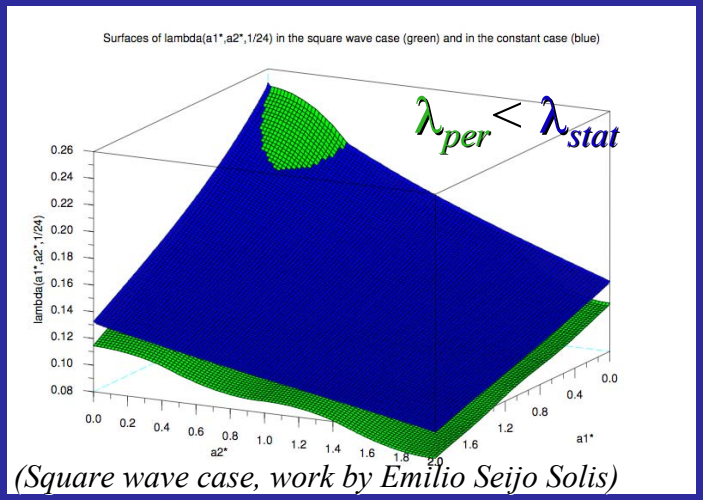
Numerical simulations have shown that if transition control functions ψ_1 on $\tilde{G}_1S$ and ψ_2 on $\tilde{G}_2M$ are of the same period 24 h and are out of phase (e.g. 0 between 0 and 12 h, and 1 between 12 and 24 h for ψ_1, with the opposite for ψ_2), then the resulting λ_{per} is alwayslower than the corresponding value λ_{stat} for $\psi_1 \square \psi_2 \square 0.5$, whatever the durations a_1, a_2 of the first 2 phases (the third one, M, being fixed as of 1 h in a total of 24 h for the whole cell cycle, with no control on M/G_1, i.e., ψ_3=1).

$(K_{i->i+1}(t,a) = \psi_i(t) \,.\, 1_{\{a \geq a_i\}}(a))$



ψ_1, ψ_2 (*sine waves or square waves in antiphase*)

$$\forall a_1 > 0, \forall a_2 > 0,$$
$$if \quad a_1 + a_2 + 1/24 = 1$$
$$then \quad \lambda_{per} < \lambda_{stat}$$



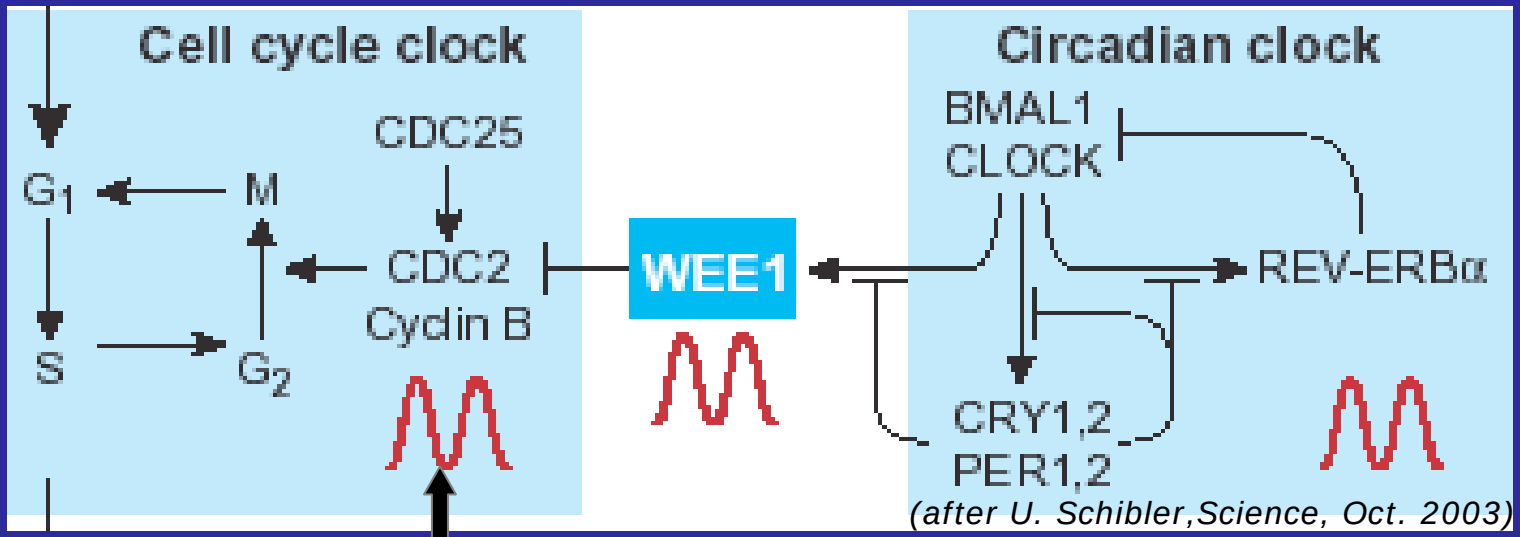
(Square wave case, work by Emilio Seijo Solis)

consistent with observations, if one assumes

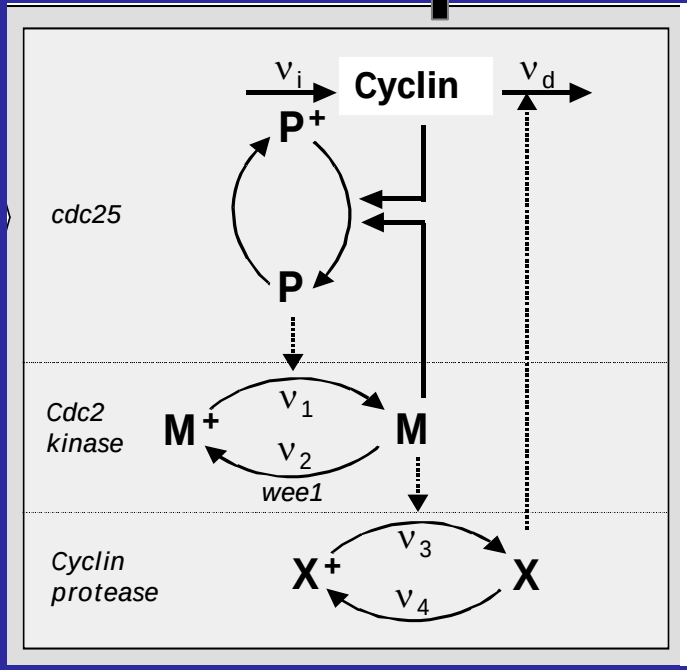
λ*(LD 12-12)* = $\lambda_{per} < \lambda_{stat}$ = λ*(jet-lag)*

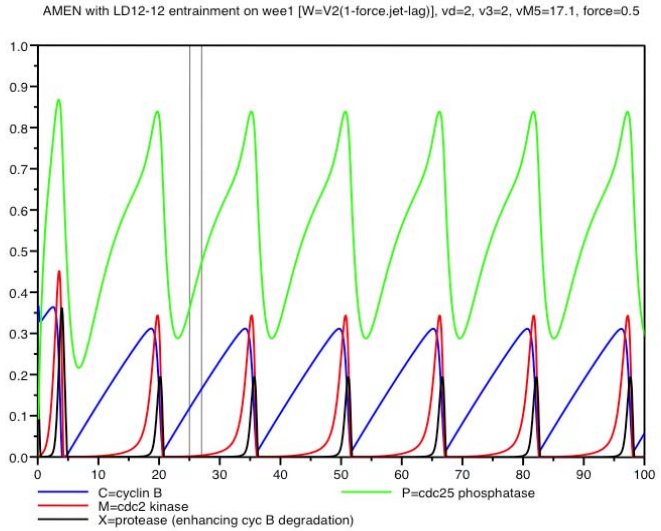
(jet-lag=desynchronisation between clocks??)

Following another explanatory track: a molecular connection between cell cycle and circadian clock: Cdk1 opens G2/M gate; Wee1 inhibits Cdk1



(after U. Schibler,Science, Oct. 2003)



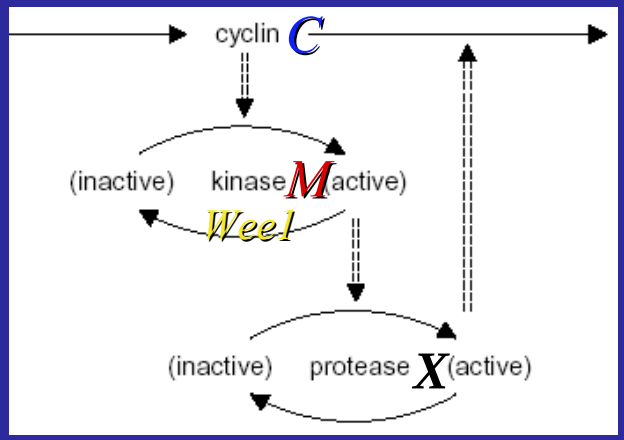


Mitotic oscillator model by Albert Goldbeter, 1997, here with circadian entrainment by a square wave standing for Wee1

Clock disruption is not necessarily to be represented by absence of control:

Connecting a circadian clock model with the cell cycle at G_2/M transition and using a disrupted clock model

Using A. Golbeter's minimal model for the G_2/M transition:



Wee1

$$\frac{dC}{dt} = v_i - k_d C - v_d X \frac{C}{K_d + C}$$

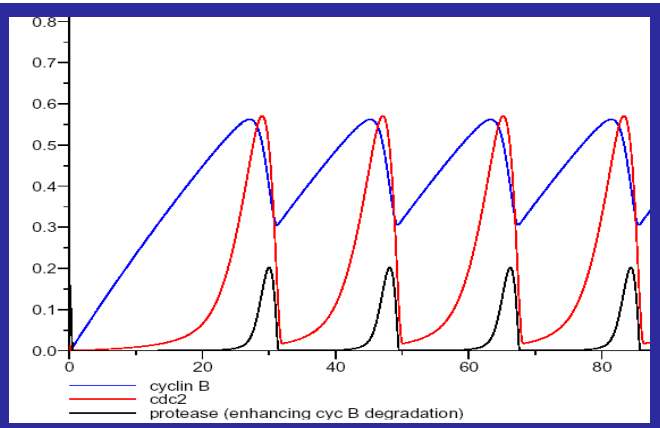
$$\frac{dM}{dt} = v_1 \frac{C}{K_c + C} \frac{(1 - M)}{K_1 + (1 - M)} - V_2 \frac{M}{K_2 + M},$$

$$\frac{dX}{dt} = v_3 M \frac{(1 - X)}{K_3 + (1 - X)} - V_4 \frac{X}{K_4 + X}.$$

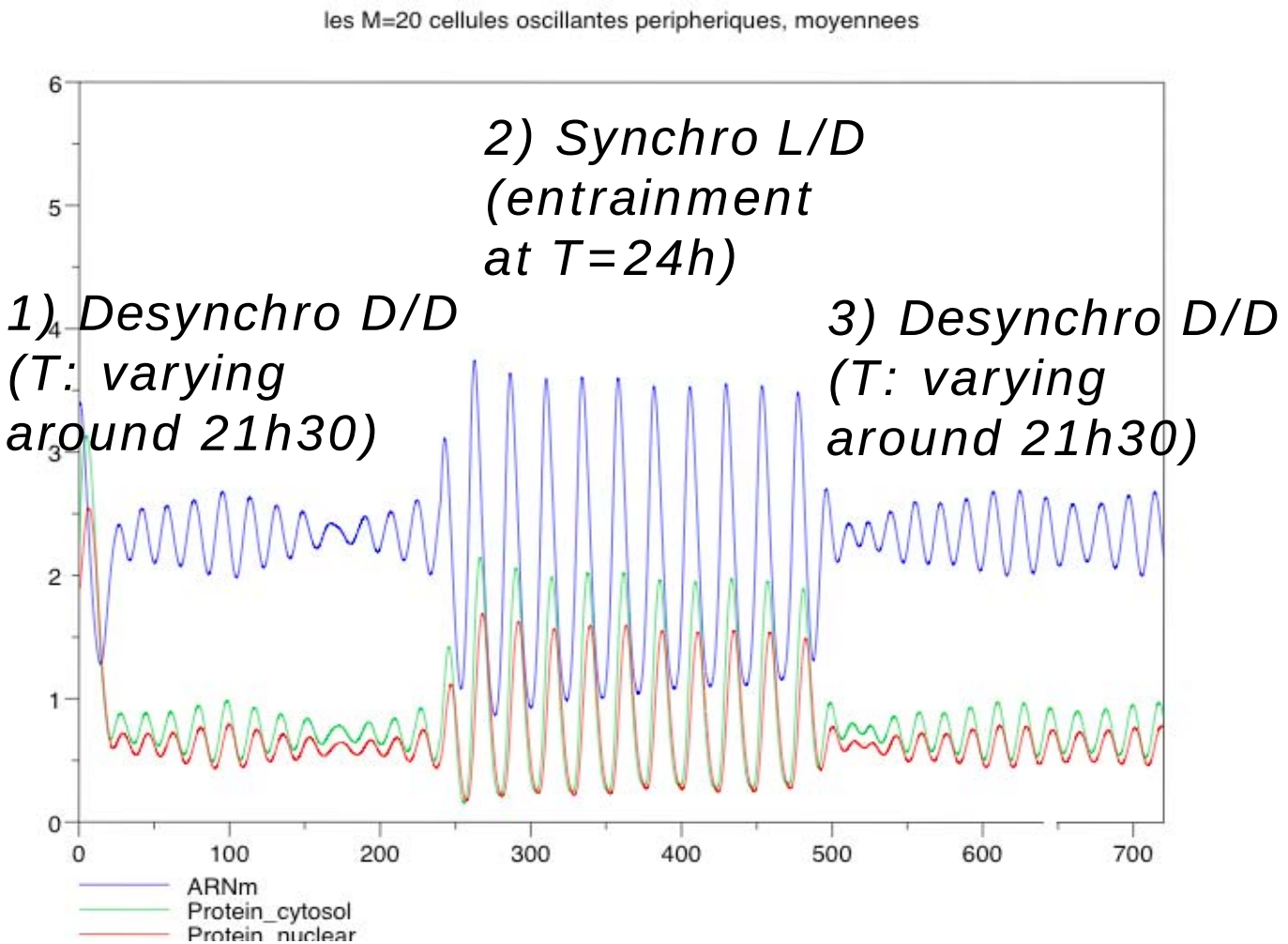
C = cyclin B, M = cyclin dependent kinase Cdk1, X = degrading protease

Input: *Bmal1=Wee1*; output: kinase M=Cdk1=ψ
Switch-like dynamics of dimer Cyclin B-Cdk1
Adapted to describe G_2/M phase transition

(A. Goldebeter Biochemical oscillations and cellular rhythms, CUP 1996)



Example of disrupted clock model: averaged *peripheral* oscillator model 1) without *central* entrainment (e.g., by light); 2) with; 3) without

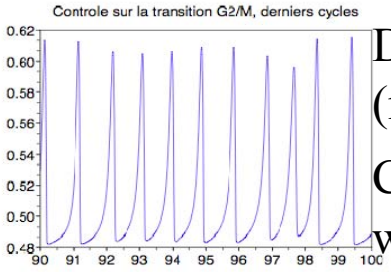


Resulting Bmal1 controls Wee1, that inhibits Cdk1= ψ, in proliferating cells

JC, Proc. IEEE-EMBC 2006, IEEE-EMB Mag 2008

Clock perturbations and cell population proliferation

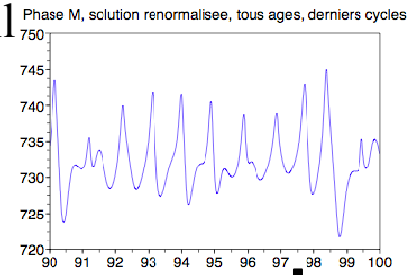
(Wee1 here identified as averaged Bmal1 in a circadian clock model)

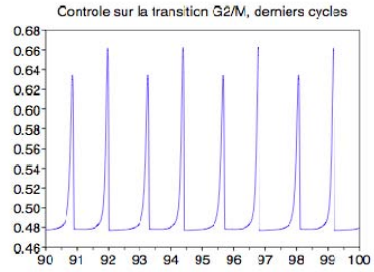


Desynchronised Wee1 (no entrainment by light):

Control Cdk1= ψ with perturbed clock

Resulting irregular cell population dynamics in M phase *(2-phase model)*

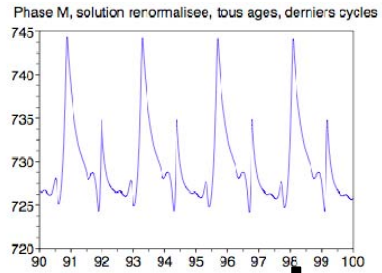




Synchronised Wee1 (entrainment by light):

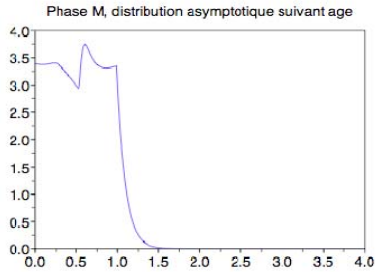
Control Cdk1= ψ with unperturbed clock

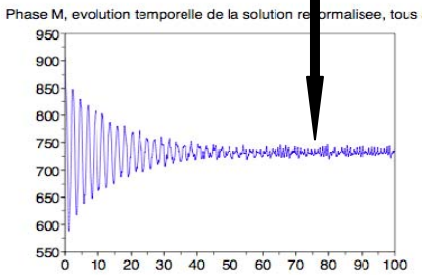
Resulting regular cell population dynamics in M phase *(2-phase model)*



Wee1=Bmal1 is desynchronised at the central (NSC) level

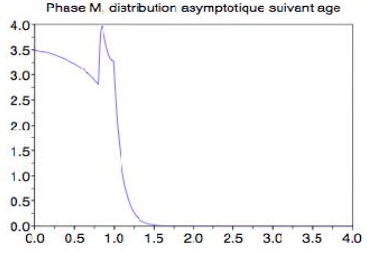
Resulting λ=0.0466

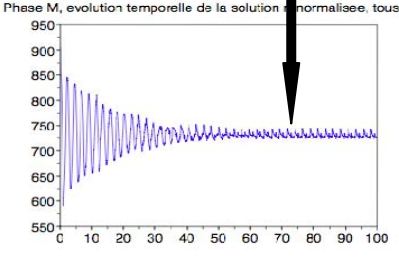




Wee1=Bmal1 is synchronised at the central (NSC) level

Resulting λ=0.0452



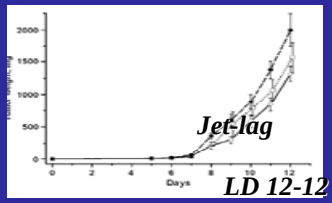


Yet another possible explanation:

Indirect action of circadian clocks on tumour growth

Underlying hypothesis:

Loss of normal physiological control on cell proliferation by circadian clocks confers a selective advantage to cancer cells by comparison with healthy cells



Possible explanation of E. Filipski's experiment (by Th. Lepoutre):

Circadian disruption is complete in healthy cells (including in lymphocytes that surround the tumour), so that the natural advantage conferred to them by circadian influence is annihilated (by contradictory messages from the central clock to proliferating healthy cells) whereas tumour cells, less (or not at all) sensitive to circadian messages, just proliferate unabashed: is such a hypothesis experimentally assessable?? …a story to be continued!

Modelling cell proliferation and quiescence

Insufficiency of linear models to accurately describe proliferation

Need for a common representation for healthy and cancer tissues

Proliferating (G_1/S/G_2/M) and quiescent (G_0) cells

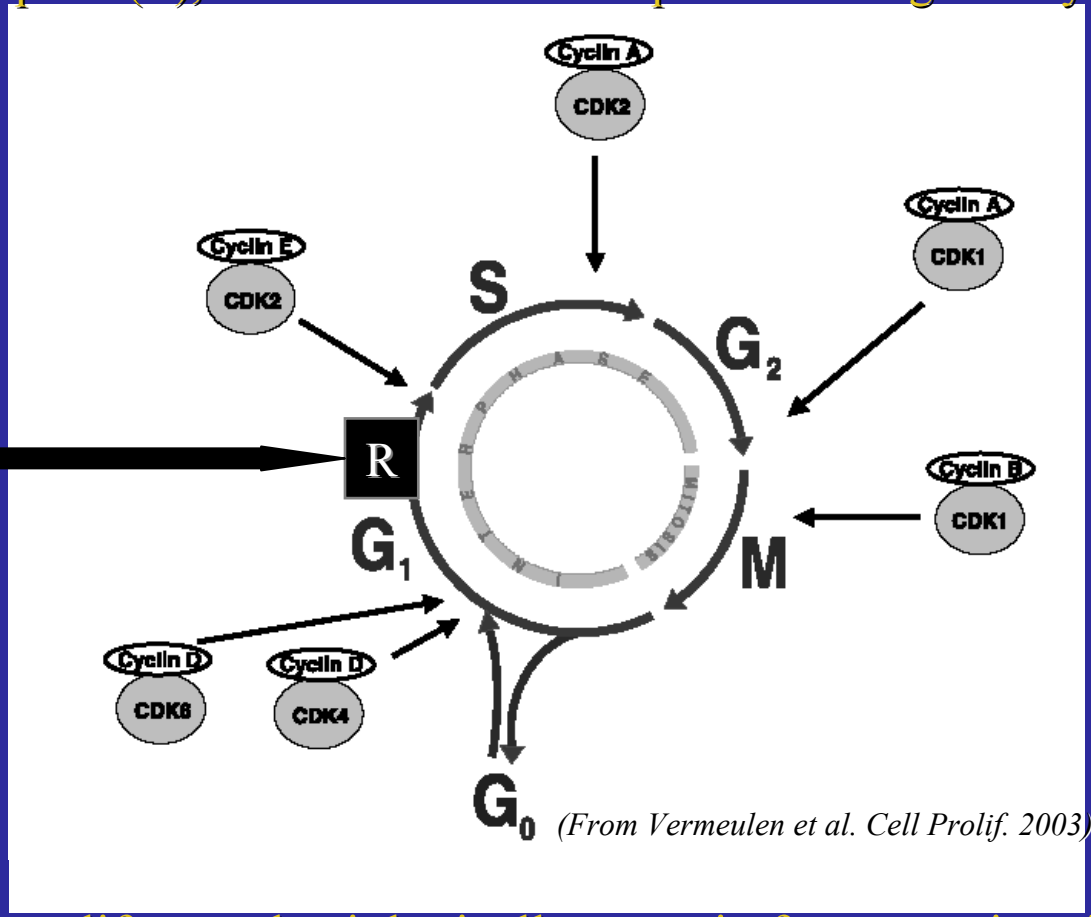
Before the restriction point (R), cells can transit from G_1 to G_0 and vice versa

After the restriction point (R), cells are committed to process through the cycle until division

after R:
mitogen-independent
progression through G_1 to S
(no return back to G_0)

Restriction point
(late G_1 phase)

before R:
mitogen-dependent
progression through G_1
(possible regression to G_0)



(From Vermeulen et al. Cell Prolif. 2003)

Most cells do not proliferate physiologically, even in fast renewing tissues

Exchanges between proliferating (G_1/S/G_2/M) and quiescent (G_0) cell compartments are controlled by mitogens and antimitogenic factors in G_1 phase

Exchanges between proliferative (p) and quiescent (q) phases: healthy and tumour tissue cases: G_0 to G_1 recruitment differs

$$\begin{cases} \dfrac{\partial}{\partial t}p(t,a,x) + \dfrac{\partial}{\partial a}\left(\Gamma_0 p(t,a,x)\right) + \dfrac{\partial}{\partial x}\left(\Gamma_1(a,x)\,p(t,a,x)\right) = \\ -\left(L(a,x) + F(a,x) + d_1\right)p(t,a,x) + G(N(t))\,q(t,a,x), \\ \dfrac{\partial}{\partial t}q(t,a,x) = L(a,x)\,p(t,a,x) - \left(G(N(t)) + d_2\right)q(t,a,x). \end{cases}$$

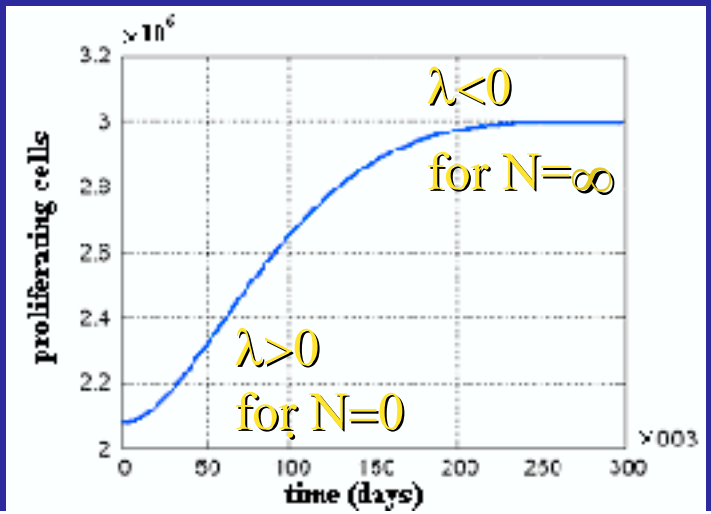
$N = \Sigma p + q$ (total number of cells at time t)

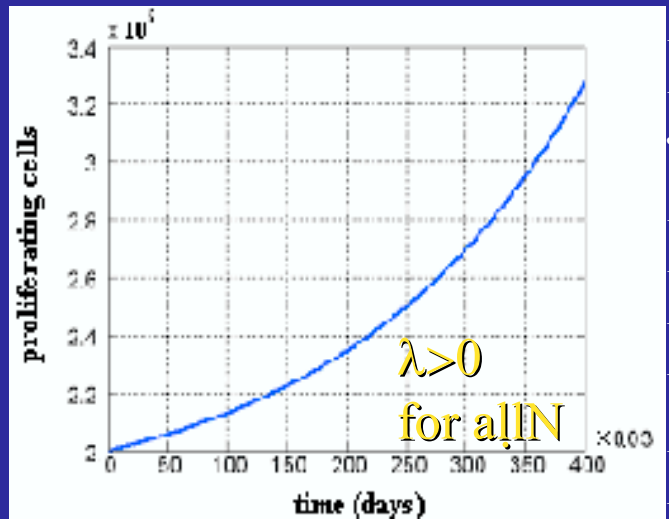
$$G(N) = \frac{\alpha_1 \theta^n}{\theta^n + N^n}$$

Healthy cells: tissue homeostasis

$$G(N) = \frac{\alpha_1 \theta^n + \alpha_2 N^n}{\theta^n + N^n}$$

Tumour cells: exponential growth





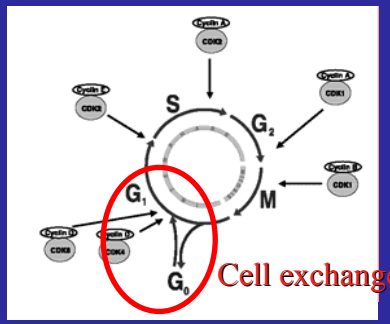
Bekkal Brikci, JC Ribba, Perthame J Math Biol 2008

Bekkal Brikci, JC Perthame Math Computer Modelling 2008

Doumic-Jauffret, MMNP 2007

Previous ODE models with two exchanging cell compartments, proliferating and quiescent

$$\begin{aligned}
\frac{dP}{dt} &= [\beta - \mu_p - r_0(N)]P + r_i(N)Q \\
\frac{dQ}{dt} &= r_0(N)P - [r_i(N) + \mu_q]Q \\
N &= P + Q, \quad P_0 + Q_0 = 1
\end{aligned}$$

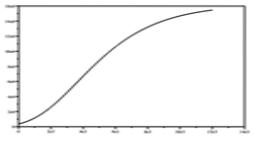


(Gyllenberg & Webb, Growth, Dev. & Aging 1989; Kozusko & Bajzer, Math BioSci 2003)

where, for instance:

$$r_0(N) = \frac{\alpha N^\gamma}{K^\gamma + N^\gamma}, \quad r_i(N) = \frac{\beta L^\delta}{L^\delta + N^\delta}$$

r_0 representing here the rate of inactivation of proliferating cells, and r_i the rate of recruitment from quiescence to proliferation



Initial goal: to justify Gompertz growth (a popular model among radiologists)

$$\frac{dx}{dt} = kx \ln\left(\frac{x_{max}}{x}\right)$$

Simple PDE models, age-structured with exchanges between proliferation and quiescence

$$\frac{\partial}{\partial t}p(t,x) + \frac{\partial}{\partial x}p(t,x) + [K(x) + \gamma(t)]p(t,x) = 0$$

$$\frac{\partial}{\partial t}q(t,x) + \frac{\partial}{\partial x}q(t,x) + [\beta(t) + \delta(t)]q(t,x) = 0$$

with :

$$p(0,x) = p^0(x),$$

$$q(0,x) = q^0(x),$$

$$p(t,0) = \beta(t)\int_0^\infty q(t,\xi)d\xi,$$

$$q(t,0) = 2\int_0^\infty K(\xi)p(t,\xi)d\xi$$

p=density of proliferating cells; *q*=density of quiescent cells; γ,δ=death terms;
K=term describing cells leaving proliferation to quiescence, due to mitosis;
β=term describing "reintroduction" (or recruitment) from quiescence to proliferation

Delay differential models with two cell compartments, proliferating (P)/quiescent (Q): *Haematopoiesis models*

(obtained from the previous model with additional hypotheses and integration in x along characteristics)

$$\frac{dP}{dt} + \gamma P - \beta(Q(t))Q(t) + \beta(Q(t-\tau))e^{-\gamma\tau}Q(t-\tau) = 0$$

$$\frac{dQ}{dt} + [\beta(Q(t)) + \delta]Q - 2\beta(Q(t-\tau))e^{-\gamma\tau}Q(t-\tau) = 0$$

$$\text{where } \beta(Q) = \frac{\beta_0\theta^n}{\theta^n + Q^n}$$

(delay τ = cell division cycle time)

(from Mackey, Blood 1978)

Properties of this model: depending on the parameters, one can have positive stability, extinction, explosion, or sustained oscillations of both populations

(Hayes stability criteria, see Hayes, J London Math Soc 1950)

Oscillatory behaviour is observed in *periodic Chronic Myelogenous Leukaemia* (*CML*) where oscillations with limited amplitude are compatible with survival, whereas explosion (blast crisis, alias *acutisation*) leads to *AML* and death

(Mackey and Bélair in Montréal; Adimy, Bernard, Crauste, Pujo-Menjouet, Volpert in Lyon)

From Adimy, Crauste, ElAbdllaoui J Biol Syst 2008 (see also: Özbay, Bonnet, JC in 47th CDC Proceedings, Cancun 2008)

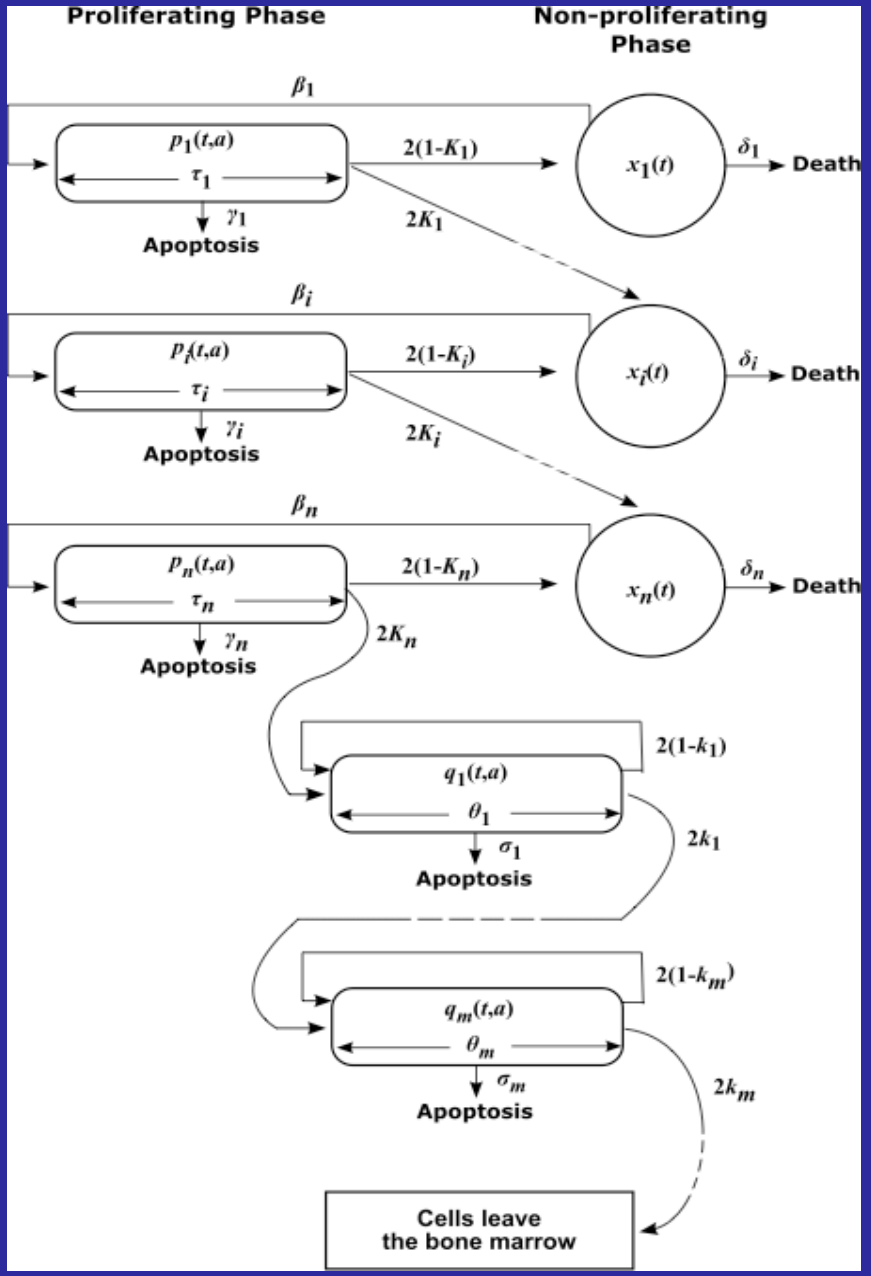
More recently (2008): modelling haematopoiesis for *Acute Myelogenous Leukaemia* (AML) …aiming at *non-cell-killing therapeutics* by inducing *re-differentiation of cells using molecules (e.g. ATRA) enhancing differentiation rates represented by K_i terms*

$$\frac{\partial r_i}{\partial t} + \frac{\partial r_i}{\partial a} = -(\delta_i + \beta_i)\, r_i, \qquad a > 0,\ t > 0,$$
$$\frac{\partial p_i}{\partial t} + \frac{\partial p_i}{\partial a} = -(\gamma_i + g_i(a))\, p_i, \qquad 0 < a < \tau_i,\ t > 0$$

where r_i and p_i represent resting and proliferating cells, respectively, with reintroduction term $\beta_i = \beta_i(x_i)$ positive decaying to zero, with population argument: $x_i(t) := \int_0^{+\infty} r_i(t,a)da$

and boundary conditions:

$$\begin{cases} r_1(t,0) = 2(1-K_1)\int_0^{\tau_1} g_1(a)p_1(t,a)da, \\ r_i(t,0) = 2(1-K_i)\int_0^{\tau_i} g_i(a)p_i(t,a)da \\ \qquad\qquad +2K_{i-1}\int_0^{\tau_{i-1}} g_{i-1}(a)p_{i-1}(t,a)da, \qquad i \geq 2, \\ p_i(t,0) = \int_0^{+\infty} \beta_i(x_i(t))r_i(t,a)da = \beta_i(x_i(t))x_i(t), \quad i \in I_n, \\ \lim\limits_{a\to+\infty} r_i(t,a) = 0. \end{cases}$$



(see *Adimy et al. JBS 2008* for more details)

Pharmacokinetics-pharmacodynamics (PK-PD): Modelling drug effects at the molecular level

Molecular PK-PD modelling in oncology

"Pharmacokinetics is what the organism does to the drug,
Pharmacodynamics is what the drug does to the organism"

- *Input*: an intravenous [multi-]drug infusion flow
- Drug concentrations in blood *and tissue* compartments (PK)
- Control of targets on the cell cycle *in tissues* (cell population PD)
- *Output*: a cell population number -or growth rate- in tumour and healthy tissues
- *Optimisation* = decreasing proliferation in tumour tissues while maintaining normal proliferation in healthy tissues

Example: 5FU (+ drug resistance) + Leucovorin

P = Plasma [5FU]

F = Intracellular [FdUMP]

Q = Plasma [LV]

L = 'Intracellular [LV]'=[CH_2THF]

N = [nrf2] efflux Nuclear Factor

A = ABC Transporter activity

S = Free [TS] (not FdUMP-bound)

B = [FdUMP-TS] binary complex

T = [FdUMP-TS-LV] irreversibl ternary complex (TS blockade)

$$\frac{dP}{dt} = -k_0 P - \frac{aP}{b+P} - l_{DPD}\frac{P}{m_{DPD}+P} + \frac{i(t)}{V}$$

$$\frac{dF}{dt} = \frac{a}{\xi}\frac{P}{b+P} - \frac{AF}{c+F} - k_1 FS + k_{-1}B$$

$$\frac{dQ}{dt} = -k_2 Q + \frac{j(t)}{V}$$

$$\frac{dL}{dt} = \frac{k_2}{\xi}Q - k_3 L - k_4 BL$$

$$\frac{dN}{dt} = \frac{\kappa F^n}{\lambda^n + F^n} - \mu N$$

$$\frac{dA}{dt} = \mu N - \nu A$$

$$\frac{dS}{dt} = -k_1 FS + k_{-1}B + \theta_{TS}(S_0 - S)$$

$$\frac{dB}{dt} = k_1 FS - k_{-1}B - k_4 BL$$

$$\frac{dT}{dt} = k_4 BL - v_T T$$

Input = LV infusion flow

Input = 5FU infusion flow

Output = blocked Thymidylate Synthase

where $l_{DPD} = l_{DPD\_BASE}\left\{1 + \varepsilon \cos\frac{2\pi(t-\varphi_{DPD})}{24}\right\}$ and $S_0 = S_{0\_BASE}\left\{1 + \delta \cos\frac{2\pi(t-\varphi_{TS})}{24}\right\}$

Simulation: 5 courses of 2 week-therapy courses

$i(t)=i_0[1+\sin\{2\pi(t-\varphi_{5FU}+9)/12\}]$ and $j(t)=j_0[1+\sin\{2\pi(t-\varphi_{LV}+9)/12\}$, then zero for 12 hours

4 days of 5FU+LV infusion,12 hours a day, every other week

P = Plasma [5FU]

F = Intracellular [FdUMP]

Q = Plasma [LV]

L = 'Intracellular[LV]'=[CH_2THF]

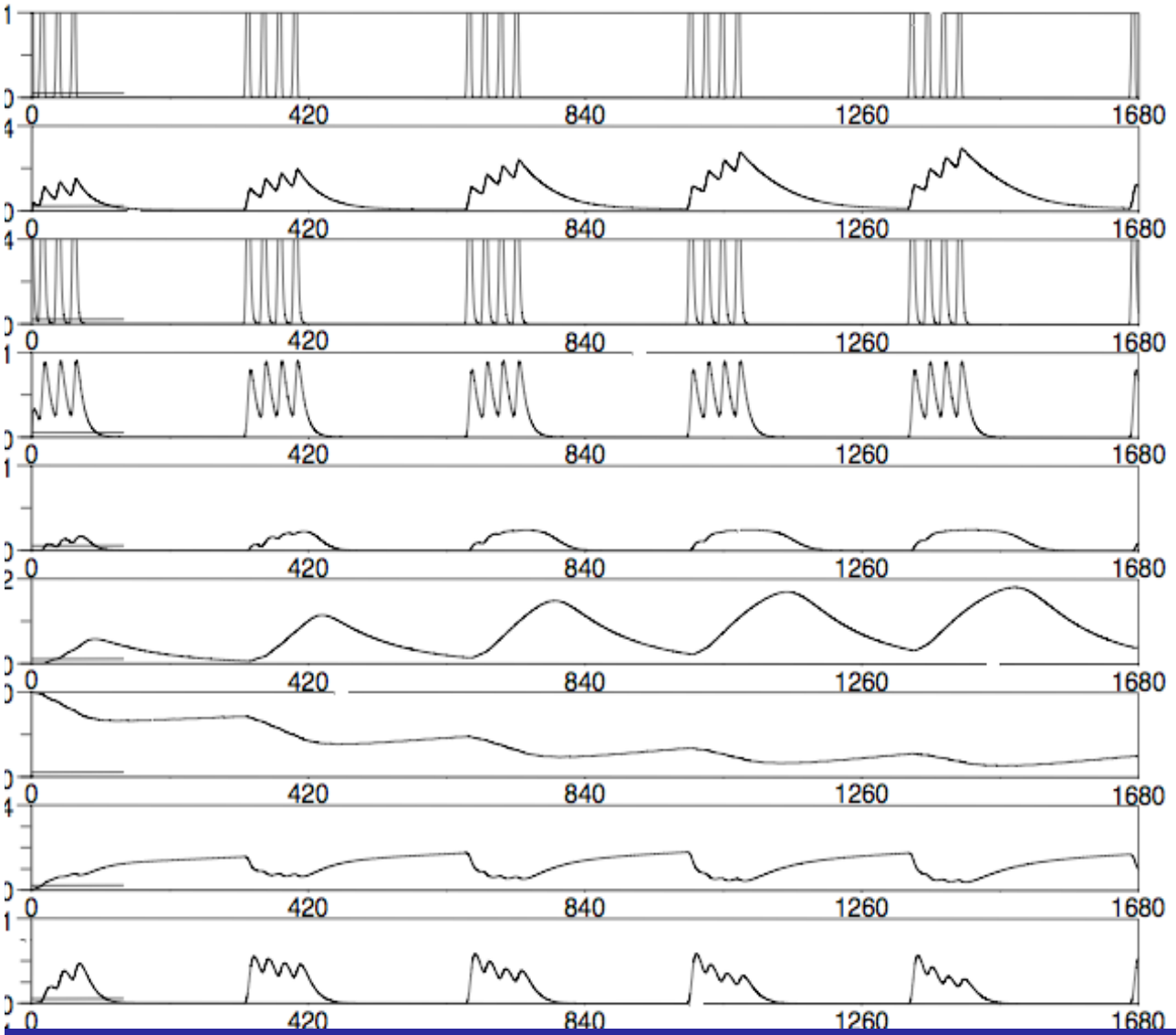
N = [nrf2] 5FU-triggered Nuclear Factor

A = ABC Transporter activity, nrf2-inducted

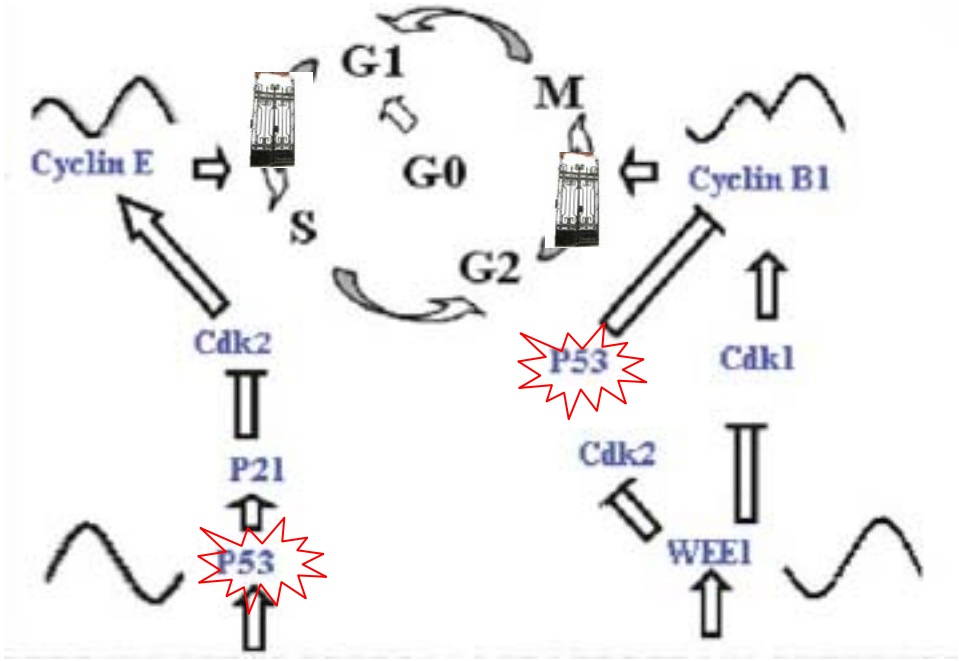
S = Free [TS] (not FdUMP-bound)

B = [FdUMP-TS] reversible binary complex

T = [FdUMP-TS-LV] stable (=irreversible) ternary complex = TS blockade



To connect PK-PD with cell proliferation: The sentinel protein p53 senses DNA damage due to cytotoxic drugs, induces cell cycle arrest and launches either DNA mismatch repair or apoptosis



(from You et al., Breast Canc Res Treat 2005)

Connecting DNA damage with cell cycle arrest at G1/S and G2/M checkpoints by inhibition of phase transition functions $\psi_{\square}$ and apoptosis (a task that still remains to be done: PhD thesis under way)

Main challenge:
Optimisation of cancer therapeutics

Optimal control of anticancer pharmacotherapy

1) *Objective function* to be minimised: cell population growth rate or cell population density in tumour tissues

2) *Control function*: instantaneous [dynamic] intravenous infusion = [multi-]drug delivery flow via external programmable pumps

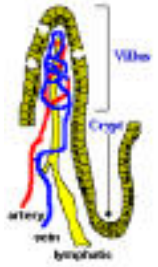
3) *Constraints* to be satisfied:
   - maintaining healthy cell population over a tolerability threshold
   - taking into account circadian phases of drug processing systems (model prerequisite)
   - *maintaining normal tissue synchronisation control by circadian clocks*
   - limiting resistances in tumour cells (*e.g. controlling induction of nrf2)*
   - others: maximal daily dose, maximal delivery flow,…

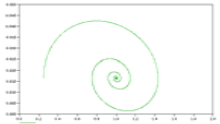
4) *With adaptation* of drug delivery flow to *patient-specific parameters* (clock phases, enzyme genetic polymorphism, target protein levels,…)

PK-PD simplified model for cancer chronotherapy (here with only toxicity constraints; target=death rate)

Healthy cells (jejunal mucosa)

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V}\Phi(t)$$
$$\frac{dC}{dt} = -\mu C + P$$
$$\frac{dZ}{dt} = -\{\alpha + f(C,t)\}Z - \beta A + \gamma$$
$$\frac{dA}{dt} = Z - Z_{eq}$$



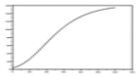


(homeostasis=damped harmonic oscillator)

(PK)

Tumour cells

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V}\Phi(t)$$
$$\frac{dD}{dt} = -\nu D + \xi_D P$$
$$\frac{dB}{dt} = \left[a \ln \frac{B_{max}}{B} - g(D,t)\right] B$$



(tumour growth=Gompertz model)

(« chrono-PD »)

$f(C,t)=F.C^{\gamma}/(C_{50}{}^{\gamma}+C^{\gamma}).\{1+\cos 2\pi(t-\varphi_S)/T\}$

$g(D,t)=H.D^{\gamma}/(D_{50}{}^{\gamma}+D^{\gamma}).\{1+\cos 2\pi(t-\varphi_T)/T\}$

Aim: balancing IV delivered drug anti-tumour efficacy by healthy tissue toxicity

(JC, Pathol-Biol 2003; Adv Drug Deliv Rev 2007)

Optimal control, step 1: deriving an objective function from the tumour cell population model

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V}\Phi(t) \quad (1)$$

$$\frac{dD}{dt} = -\nu D + P \quad (2)$$

$$\frac{dB}{dt} = \left[a \ln \frac{B_{max}}{B} - g(D,t)\right] B \quad (3)$$

Φ characteristic function of the allowed infusion time intervals

Eradication strategy: minimise $G_B(i)$, where:

$$G_B(i) = \min_{t \in [t_1, t_f]} B(t,i)$$

Argument i of objective function G_B is the drug infusion flow, some function in $L^2([t_0,t_f])$

or else:

Stabilisation strategy: minimise $G_B(i)$, where:

$$G_B(i) = \max_{t \in [t_1, t_f]} B(t,i)$$

($t_1 < t_f$ being some fixed observation time after t_0, beginning of infusion interval)

Optimal control, step 2: deriving a constraint function from the enterocyte population model

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V}\Phi(t) \quad (1)$$

$$\frac{dC}{dt} = -\mu C + P \quad (2)$$

$$\frac{dZ}{dt} = -\{\alpha + f(C,t)\}Z - \beta A + \gamma \quad (3)$$

$$\frac{dA}{dt} = Z - Z_{eq} \quad (4)$$

Minimal toxicity constraint, for $0 < \tau_A < 1$ (e.g. $\tau_A = 50\%$):

$$F_A(i) = \tau_A - \min_{t \in [t_0, t_f]} A(t,i)/A_e \leq 0$$

±other possible constraints: $\max_{t \in [t_0, t_f]} i(t) \leq i_{max}$, $\int_{t_0}^{t_f} i(t) \leq AUC_{max}$

Optimal control problem: defining a lagrangian:

$$\mathcal{L}(i,\theta) = G_B(i) + \theta F_A(i)\,,\ \text{where}$$

$$0 \le i \le i_{max}\,,\ i \in L^2([t_0,t_f]),\ \int_{t_0}^{t_f} i(t) \le AUC_{max}\,,\ \text{and}\ \theta \ge 0$$

then:

$$\min_{F_A(i)\le 0} G_B(i) = \min_{i \in L^2([t_0,t_f])} \max_{\theta \ge 0} \mathcal{L}(i,\theta)$$
$$\pm\ other\ constraints$$

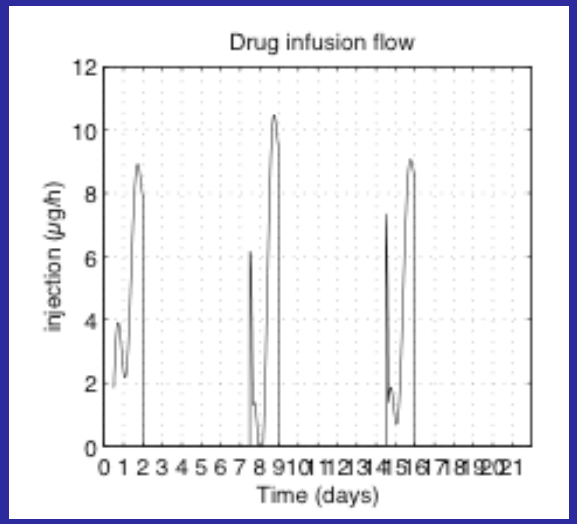
If G_B and F_A were convex, then a necessary and sufficient condition would be:

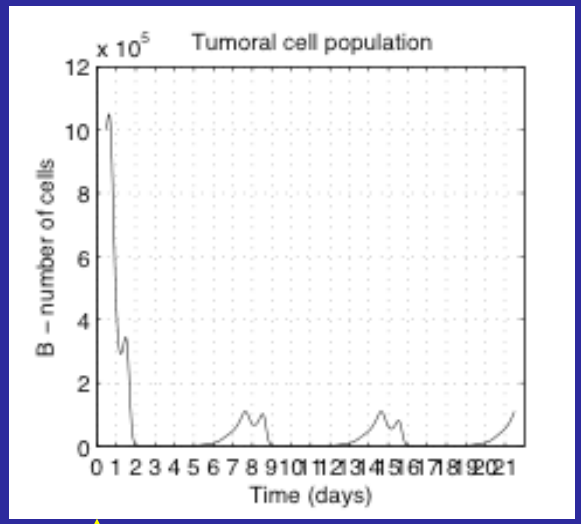
$$\min_i \max_{\theta>0} \mathcal{L}(i,\theta) = \max_{\theta>0} \min_i \mathcal{L}(i,\theta)$$

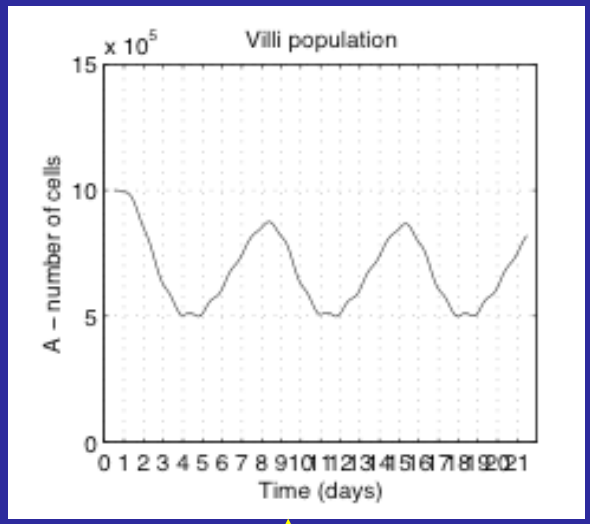
…i.e. the minimum would be obtained at a saddle point of the lagrangian, reachable by a Uzawa-like descent algorithm; in general, this yields only sufficient conditions, i.e. we may miss minima of the objective function G_B. Nevertheless:…

Optimal control: results of a tumour stabilisation strategy using this simple one-drug PK-PD model

(and investigating more than Uzawa's algorithm fixed points, by storing best profiles)







Objective: *minimising the maximum of the tumour cell population*

Constraint : *preserving the jejunal mucosa according to the patient's state of health*

Result : optimal infusion flow i(t) adaptable to the patient's state of health

(according to a tunable parameter τ_A: here preserving τ_A=50% of enterocytes*)*

(C. Basdevant, JC, F. Lévi, M2AN 2005; JC Adv Drug Deliv Rev 2007)

More challenges and future prospects

Individualised treatments in oncology

Genetic polymorphism: between-subject variability
for pharmacological model parameters

- According to subjects, different expression and activity levels of drug processing enzymes and proteins (uptake, degradation, active efflux, e.g. GST□, DPYD, UGT1A1, P-gp,…)
  and drug targets (e.g. Thymidylate Synthase, Topoisomerase I)

- The same is true of DNA mismatch repair enzyme gene expression (e.g., ERCC1, ERCC2)

- More generally, pharmacotherapeutics should be guided more by molecular alterations of the DNA than by location of tumours: genotyping patients with respect to anticancer drug processing may become the rule in oncology in the future *(see e.g. G. Milano & J. Robert in Oncologie 2005)*

Other frontiers in cancer therapeutics

1. *Immunotherapy*:

   Not only using cytokines and actual anticancer vaccines, but also examining delivery of cytotoxics from the point of view of their action on the immune system

   (Review by L. Zitvogel in Nature Rev. Immunol. 2008)

2. *The various facets of (innate/acquired/(ir)reversible) drug resistance*:

- Repair enzymes, mutated p53: cell cycle models with by-pass of DNA damage control
- ABC transporters, cellular drug metabolism: molecular PK-PD ODEs (or PDEs)
- Microenvironment, interactions with stromal cells: competition/cooperativity models
- Mutations of the targets: evolutionary game theory, evolutionary dynamics models

3. *Developing non-cell-killing therapeutic means*:

- Associations of cytotoxics and redifferentiating agents (e.g. retinoic acid in AML3)
- Modifying local metabolic parameters? (pH) to foster proliferation of healthy cells

Collaborators

INRIA Bang project-team: *Annabelle Ballesta, Fadia Bekkal Brikci, Luna Dimitrio, Marie Doumic, Herbert Gayrard, Thomas Lepoutre, Benoît Perthame, Emilio Solis*

Other INRIA project-teams: *François Fages (*Contraintes), *Catherine Bonnet (*Disco*), Stéphane Gaubert* (Maxplus), *Jean-Charles Gilbert* (Estime), *Mostafa Adimy (*Dracula*)*

INSERM U 776 "Biological Rhythms and Cancers" (*F. Lévi*, Paul-Brousse hospital, Villejuif): Solid tumours of Mice and Men, chronotherapeutics of colorectal cancer

UMRs UPMC- INSERM U 872 Team 18 "Resistance and survival of tumour cells" (*J.-P Marie,* Cordeliers Research Centre and Hôtel-Dieu hospital, Paris): Acute leukaemias

Université Paris-Nord (*Claude Basdevant)*: Optimal control theory and algorithms

Université Lyon 1, UMR CNRS 5208 (*Vitaly Volpert*): Haematopoiesis

past ARC INRIA ModLMC: http://www.math.u-bordeaux1.fr/~adimy/modlmc/
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past FP6 MCRTN M3CSTGT: http://calvino.polito.it/~mcrtn/
present FP7 ERASysBio C5Sys: http://www.erasysbio.net/index.php?index=272