Modelling circadian and pharmacological controls on the cell cycle and tumour growth

Jean Clairambault

INRIA Bang project-team, Rocquencourt, France
&
INSERM U 776 « Biological Rhythms and Cancers », Villejuif, France

http://www-rocq.inria.fr/bang/JC/Jean_Clairambault.html

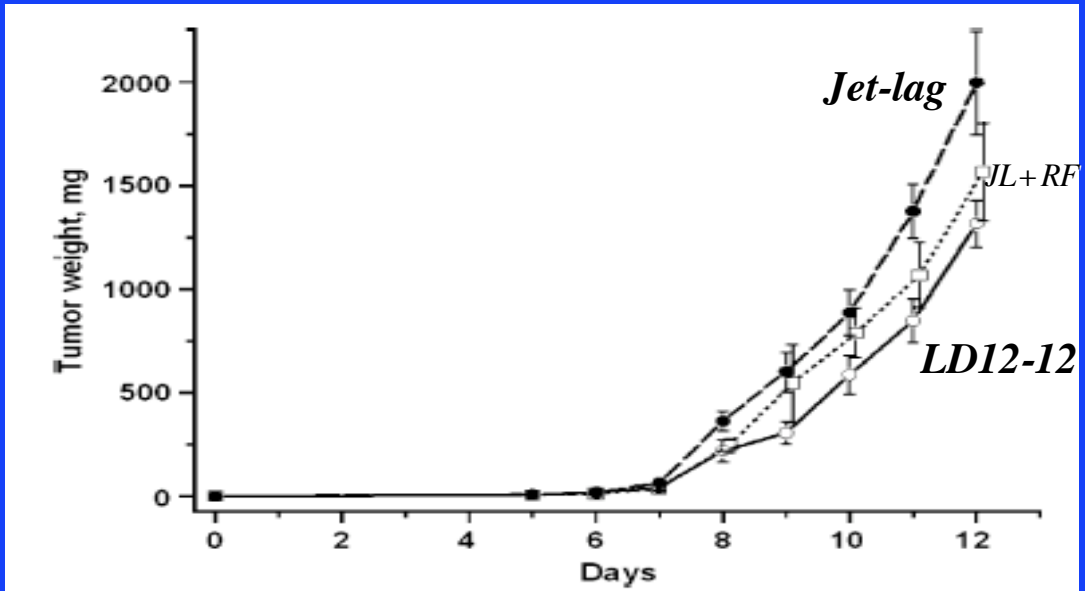
Outline of the talk

- Questions on the cell cycle and its control by the circadian clock
- Modelling periodic control on the cell cycle
- Modelling the circadian system and its disruptions
- Modelling circadian pharmacokinetics-pharmacodynamics (PK-PD)
  …at the molecular level
- Toward practical medical applications:
  a rationale for cancer chemotherapy optimisation

Questions

1) 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



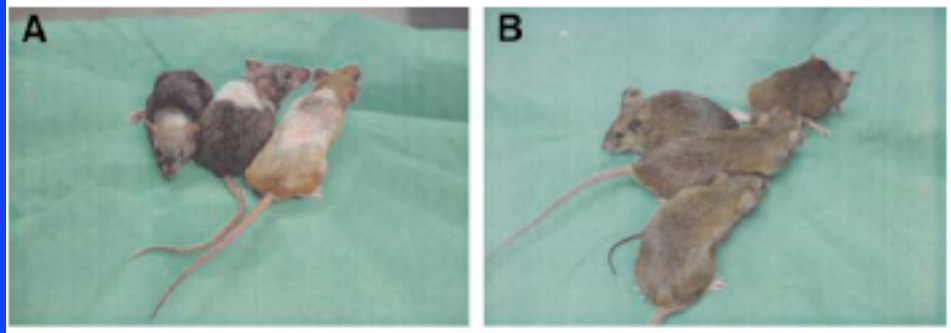
Filipski JNCI 2002, Canc. Res. 2004, JNCI 2005, Canc. Causes Control 2006

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)

2) Pathology: insights from molecular biology in mice



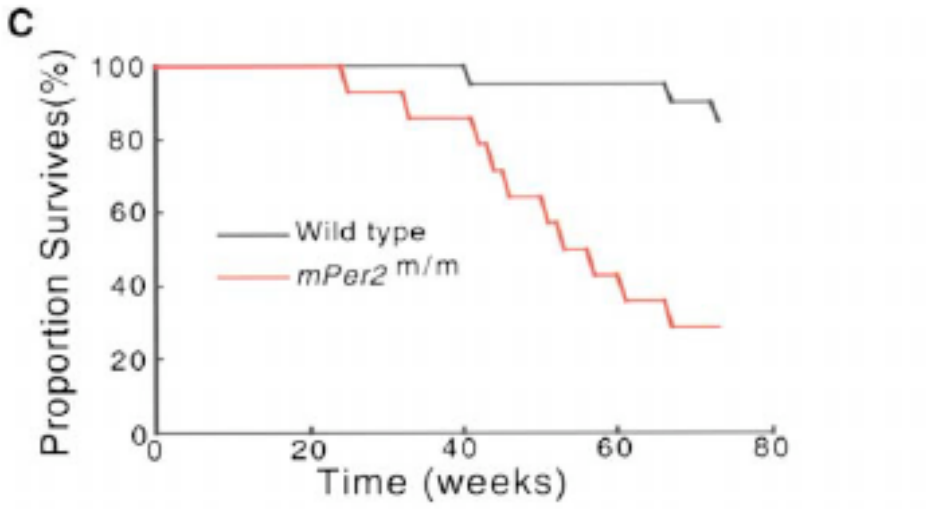


Figure 2. *mPer2*$^{m/m}$ Mice Show Increased Sensitivity to γ Radiation
(A) All the irradiated *mPer2*$^{m/m}$ mice show hair graying at 22 weeks after irradiation. Some of them also show hair loss on the back.
(B) Wild-type mice at 22 weeks after irradiation.
(C) Survival curve for wild-type and *mPer2*$^{m/m}$ mice after irradiation.

(from Fu et al., Cell 2002)

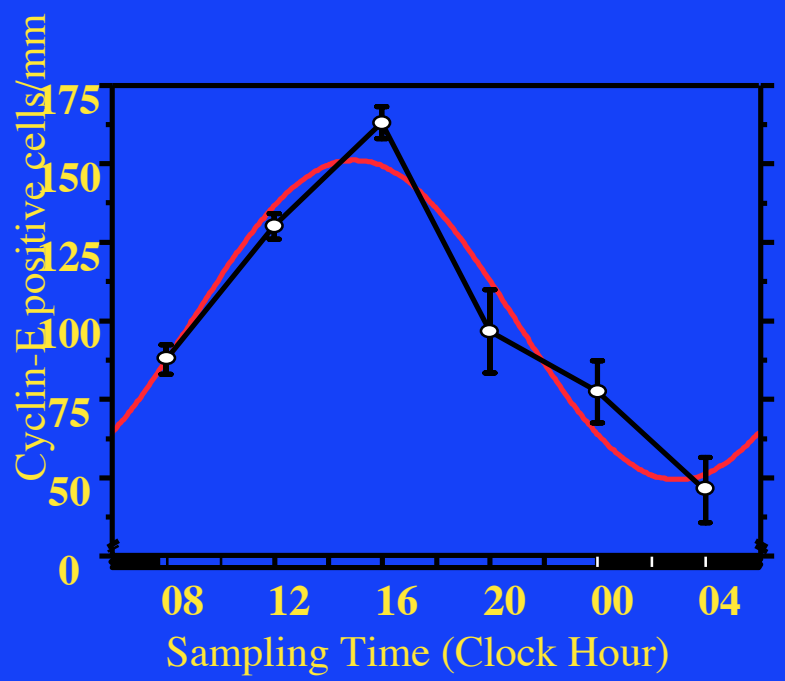
NB: Per2 is a gene of the circadian clock that has been found in all nucleated cells

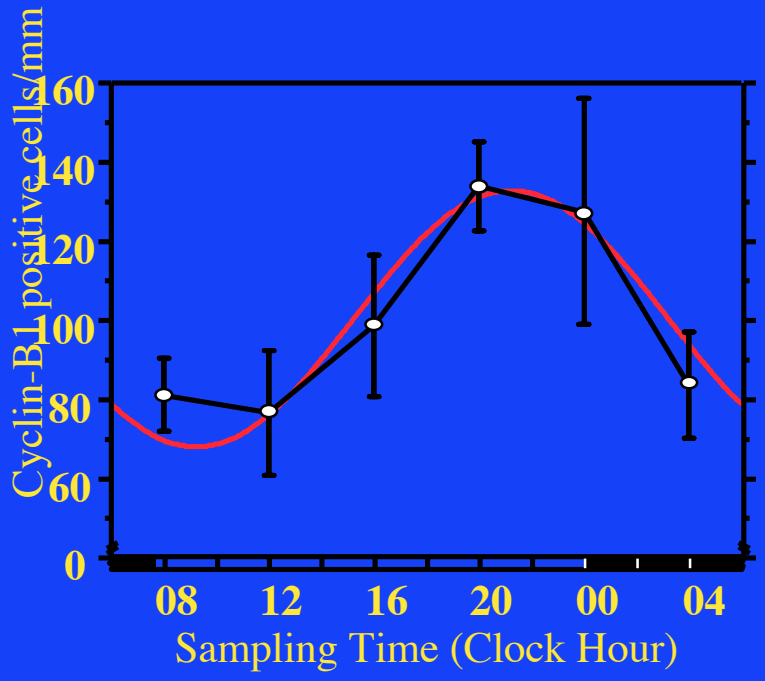
Per2$^{-/-}$ mice are more prone to develop (various sorts of) cancer following γ-irradiation than wild type mice

Hypothesis: loss of control of cell proliferation by circadian clock genes confers a selective advantage to cancer cells by comparison with healthy cells

3) 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)

4) Circadian rhythms and cancer chronotherapeutics

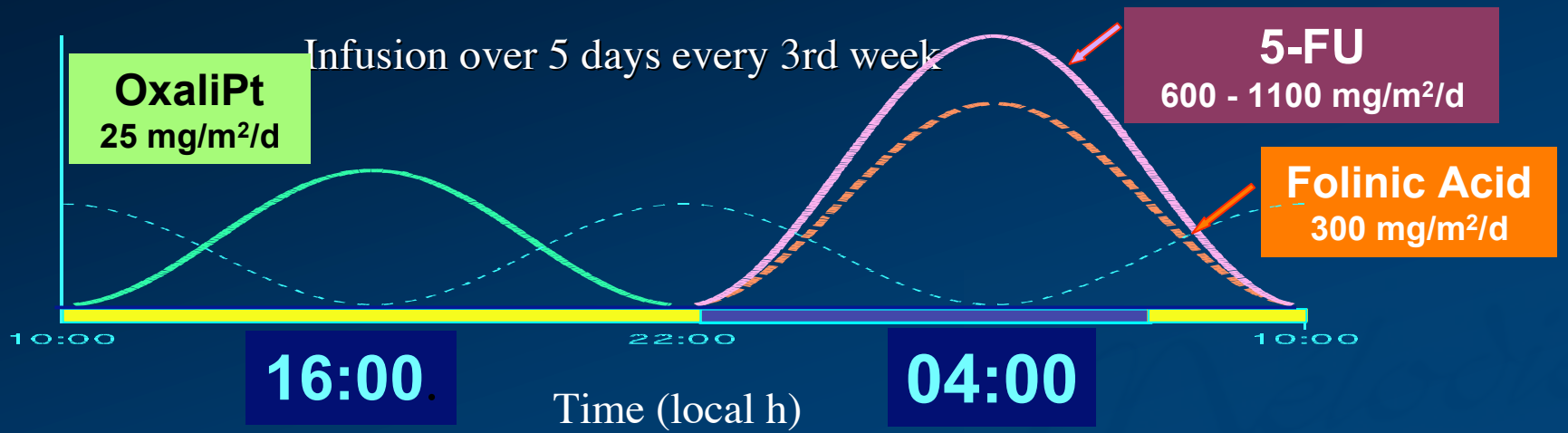
(Results from Francis Lévi's INSERM team U 776, Villejuif, France)

Improvements in response to treatment and survival of patients with colorectal cancer have been obtained by chronotherapy (i.e., drug delivery according to 24 h-rhythmic time schedules)

Patients with disrupted circadian rhythms (plasma cortisol, central temperature, rest-activity alternations) are less responsive to treatment and of poorer prognosis

Chronotherapeutics today for the treatment of colorectal cancer

Time-scheduled delivery regimen for metastatic CRC



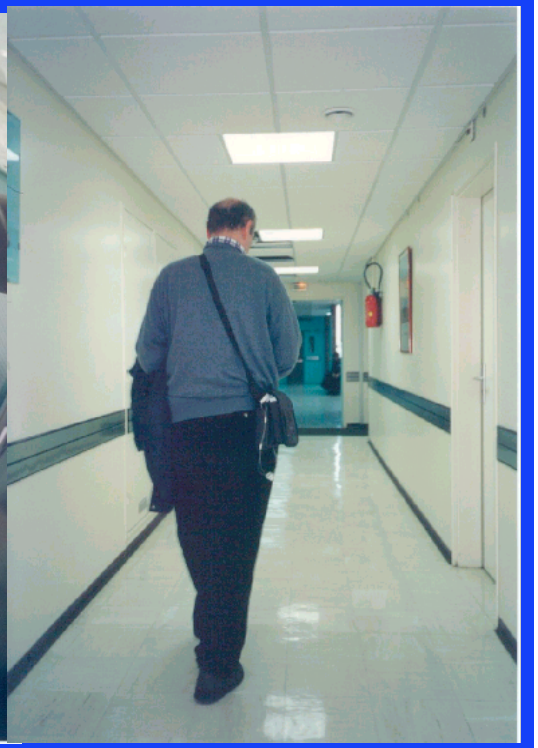
Multichannel programmable ambulatory injector for intravenous drug infusion (pompe Mélodie, Aguettant, Lyon, France)

Can such drug delivery schedules be improved?

Chronotherapy technology

Multichannel pump for chronotherapy

- Centralised programmation
- Any modulation of delivery rate
- 4 reservoirs (100-2000 mL)
- 2 independent channels
- Rates from 1 to 3000 mL/h

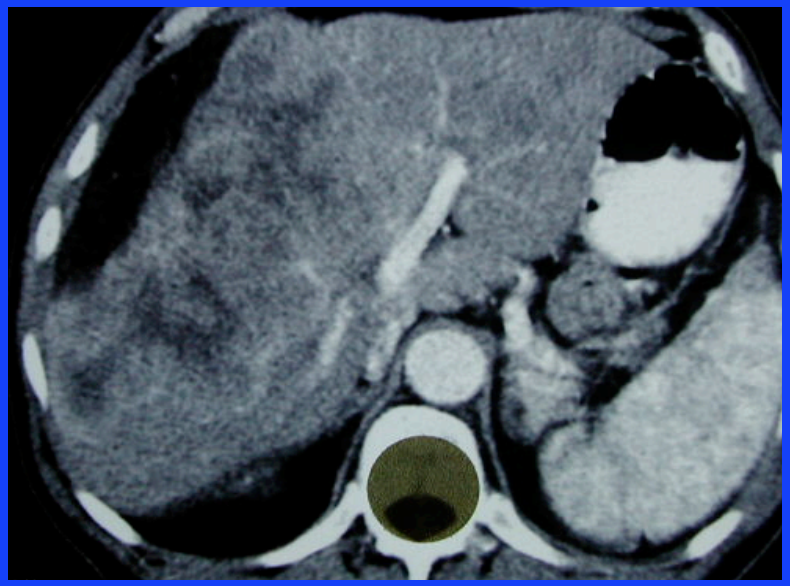


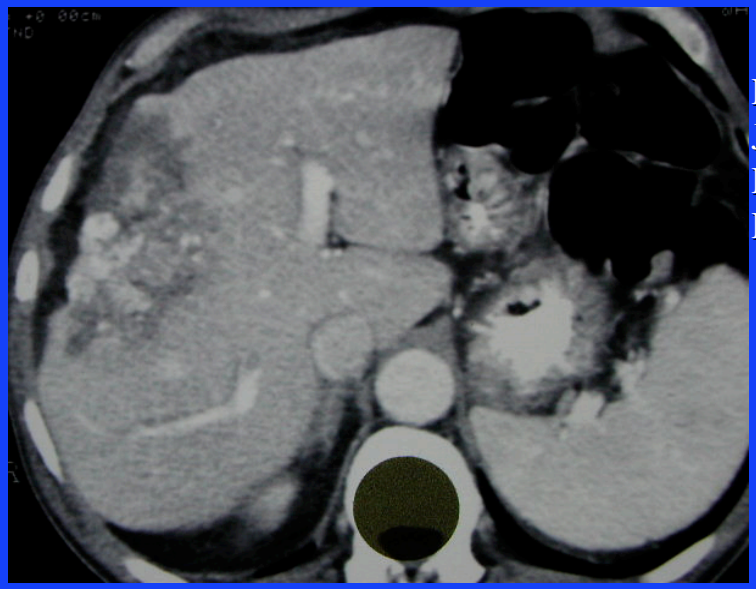
Images from the Chronotherapy Unit, Paul-Brousse Hospital, Villejuif, France

Over 2000 cancer patients registered in clinical Phase I, II or III trials

Results of cancer chronotherapy

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



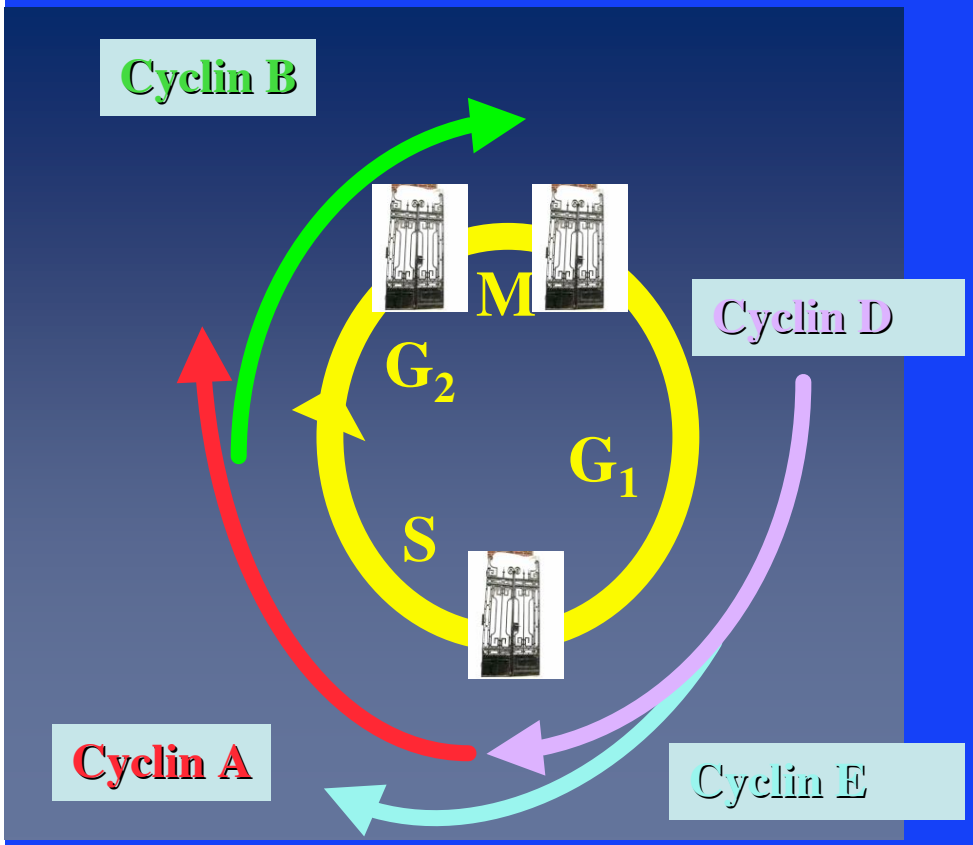


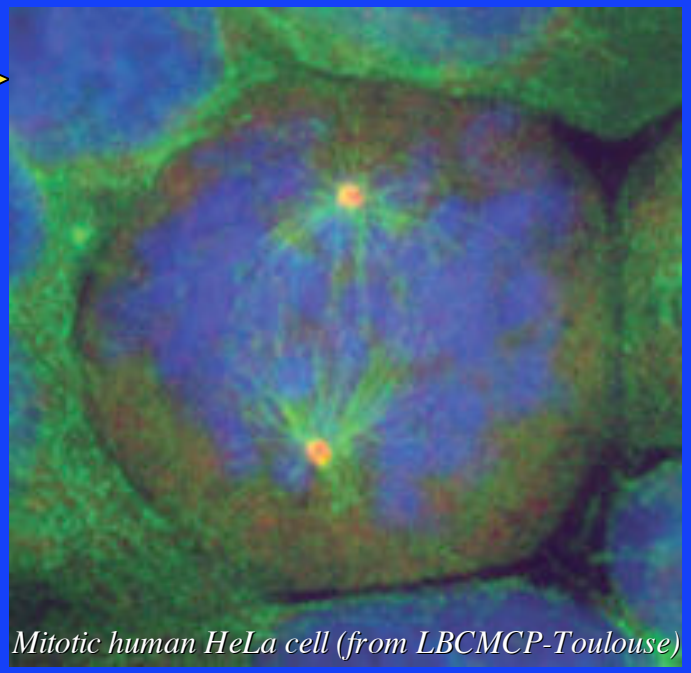
Lévi et al.
JNCI 1994 ;
Lancet 1997 ;
Lancet Onc 2001

Modelling periodic control on the cell cycle

At the origin of tumour proliferation: the cell division cycle

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



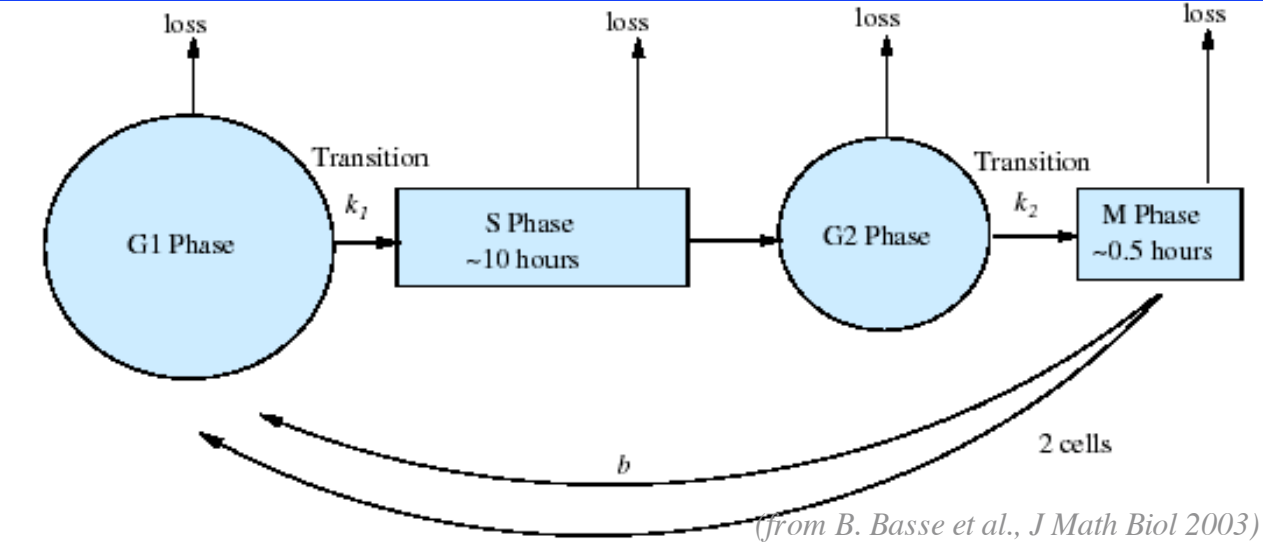


Mitotic human HeLa cell (from LBCMCP-Toulouse)

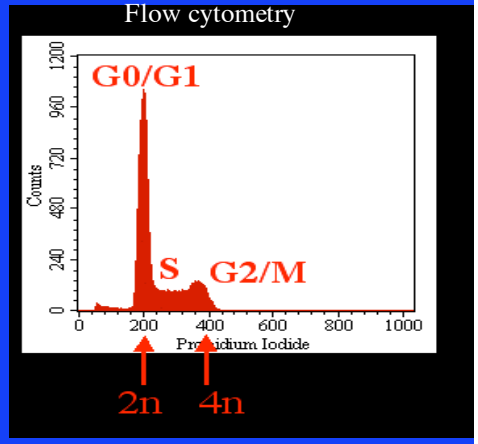
Physiological / therapeutic control
- on transitions between phases (G_1/S, G_2/M, M/G_1)
- on death rates inside phases (apoptosis or necrosis)
-on the inclusion in the cell cycle (G_0 to G_1 recruitment)

Modelling the cell division cycle

Age-structured PDE models for cycling cell populations



(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, with age a at time t;
d_i:=death rate;
$K_{i-1->i}$:=transition rate (with a factor 2 for $i=1$)
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") 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]

Clairambault, Laroche, Mischler, Perthame RR INRIA #4892, 2003

According to the Krein-Rutman theorem (infinite-dimensional form of the Perron-Frobenius theorem), there exists a nonnegative first eigenvalue λ such that, if $\widetilde{N}_i(t,a) = e^{-\lambda t} n_i(t,a)$, then there exist N_i , bounded solutions to the problem:

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

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

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

the φ_i being solutions to the dual problem; this can be proved by using a generalised entropy principle (GRE). Moreover, if the control (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.

Michel, Mischler, Perthame, C. R. Acad. Sci. Paris Ser. I (Math.) 2004; J Math Pures Appl 2005

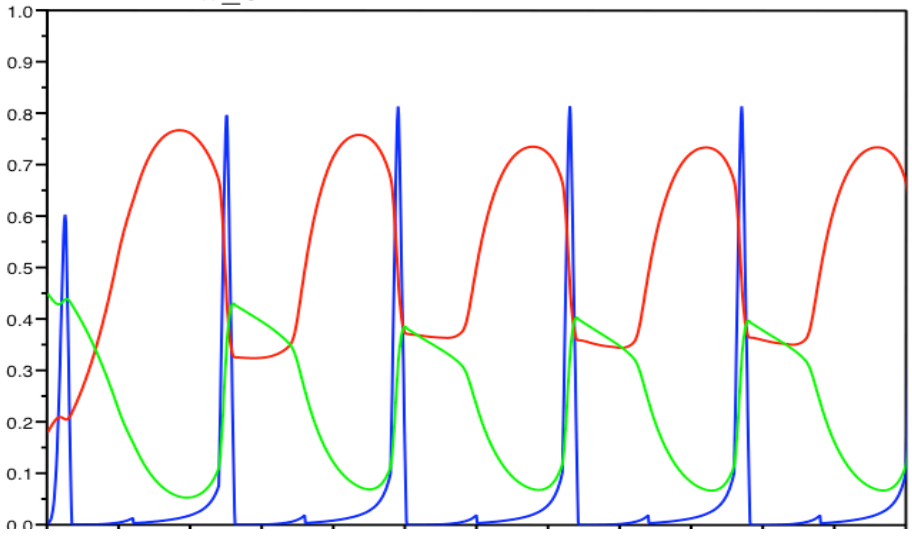
Clairambault, Michel, Perthame C. R. Acad. Sci. Paris Ser. I (Math.) 2006; Proc. ECMTB Dresden 2005, Birkhäuser 2007

To sum up: a growth exponent for the cell population

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 asymptotically (i.e., for large times) 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)

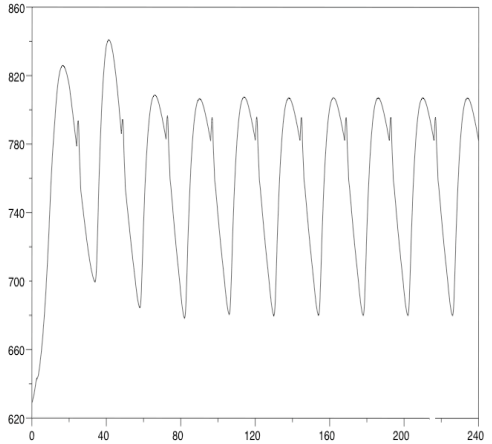
$$\mathcal{N}_i^{tot}(t) = e^{-\lambda t} \int_{\alpha \geq 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$



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

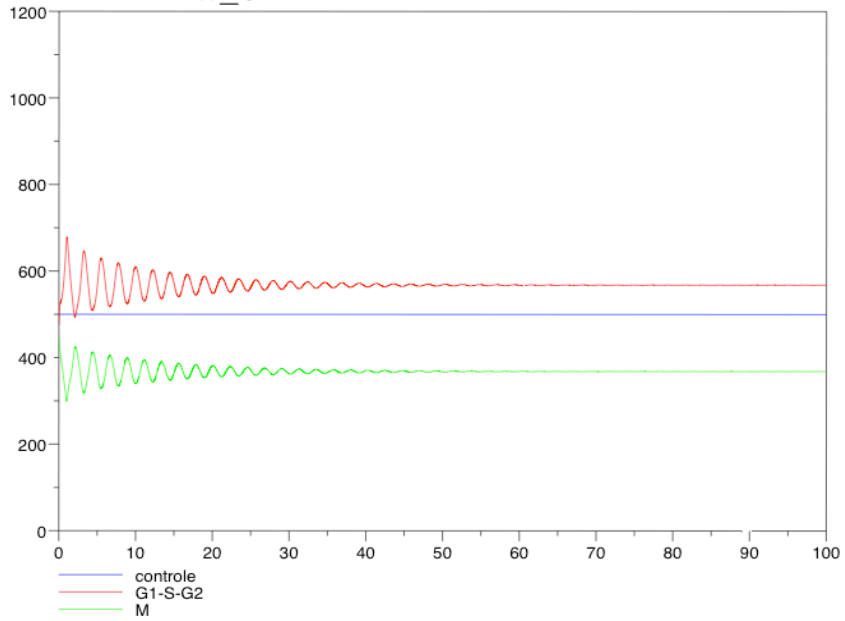
Entrainment of the cell division cycle by CDK1 at the circadian period

All cells, surfing on the exponential growth curve



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

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

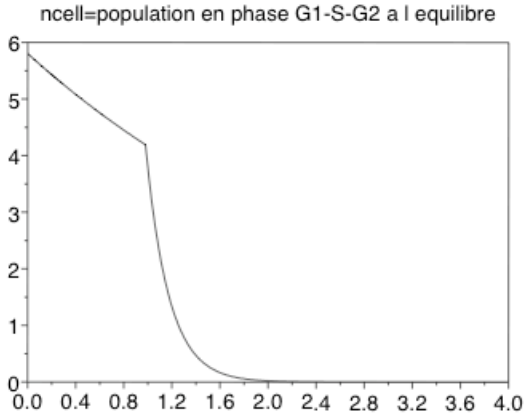


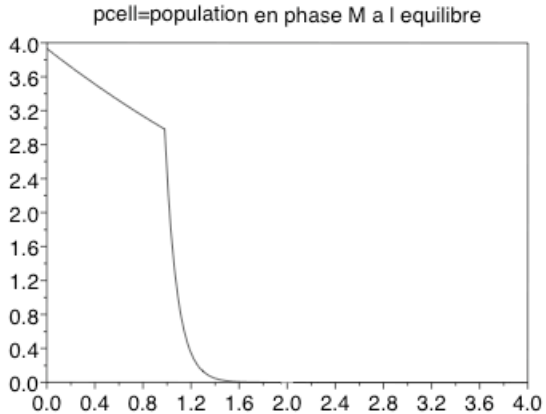
The total population of cells

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

inside each phase follows asymptotically an exponential behaviour

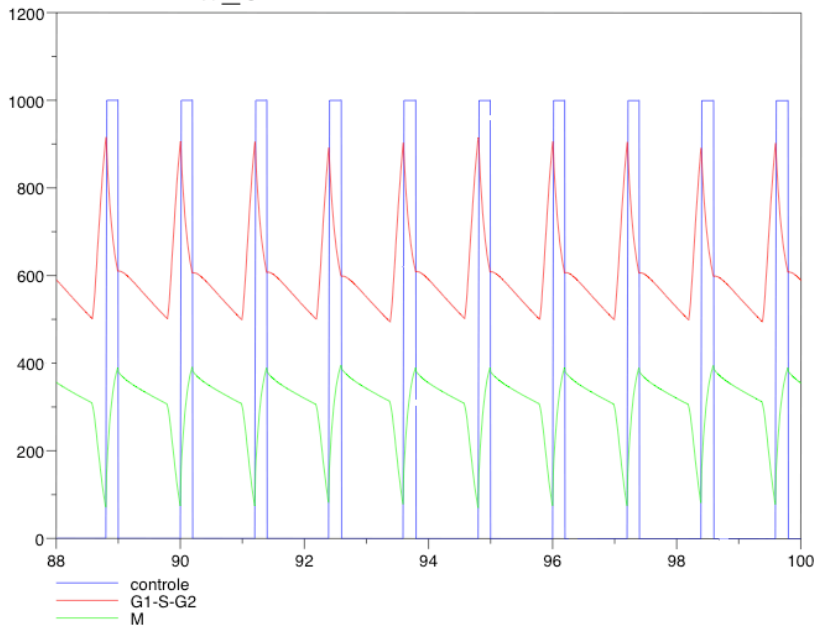
Stationary state distribution of cells inside phases according to age *a:*
no control -> exponential decay





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

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

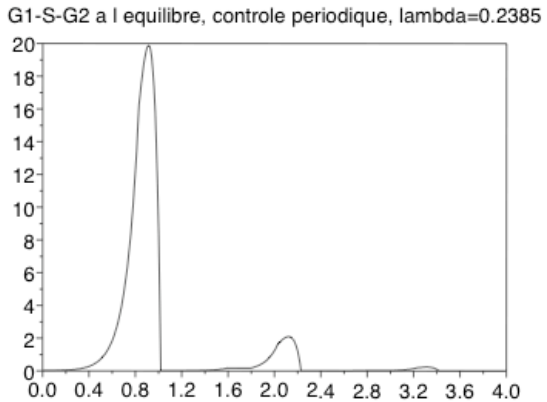


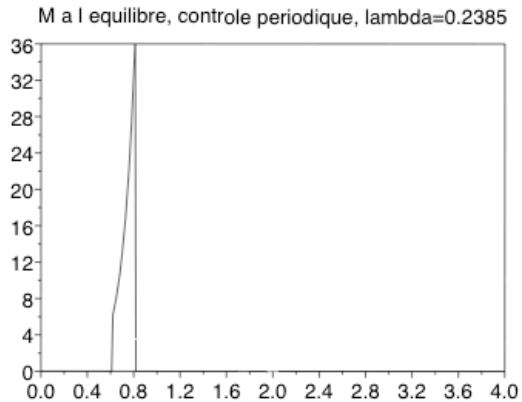
The total population of cells

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

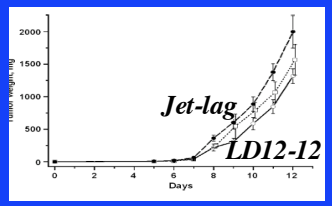
inside each phase follows asymptotically an exponential behaviour *tuned by a periodic function*

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





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:

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

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

Clairambault, Michel, 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

(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 if one assumes that

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

Clairambault, Michel, 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

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

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) time ratio, ψ_1	(periodic) λ_{per}	(constant) 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

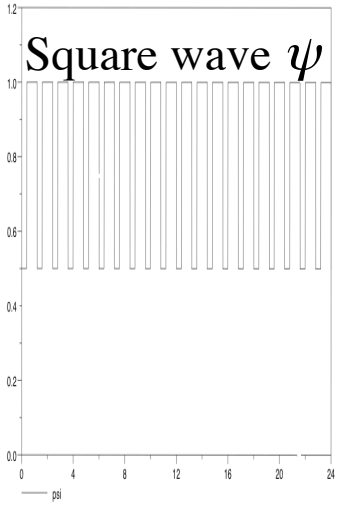
ψ_2: G2/M gate open 12 h / closed 12 h

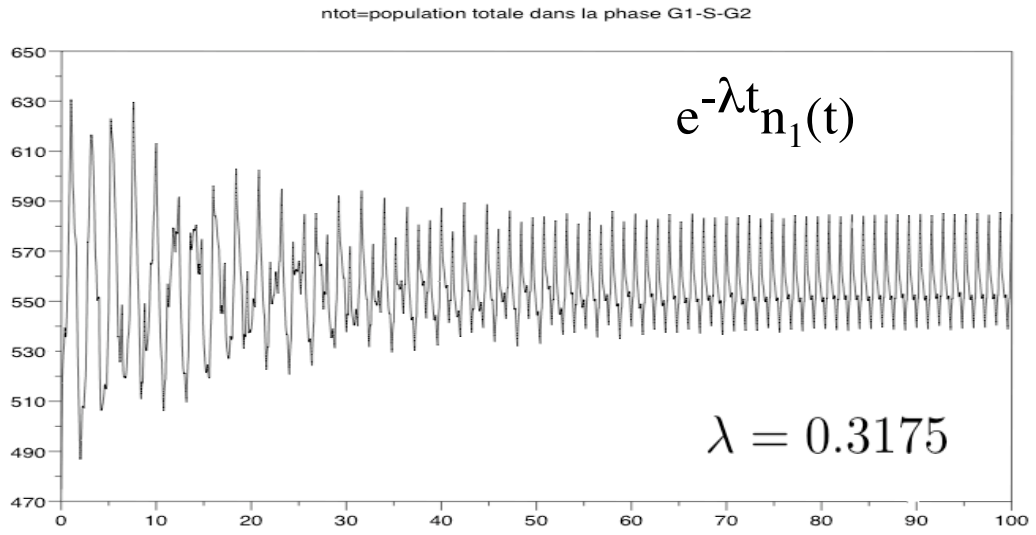
(G1-S-G2/M) time ratio, ψ_2	(periodic) λ_{per}	(constant) λ_{stat}
1	0.2623	<u>0.2821</u>
2	0.3265	<u>0.3448</u>
3	...	...
4	...	...
5	...	...
6	...	...
7	0.4500	<u>0.4529</u>
8	<u>0.4588</u>	0.4575
10	<u>0.4713</u>	0.4641
20	<u>0.5006</u>	0.4818

Clairambault, Michel, Perthame C. R. Acad. Sci. Paris Ser I (Math.) 2006; Proceedings ECMTB Dresden 2005, Bikhäuser, to appear 2007

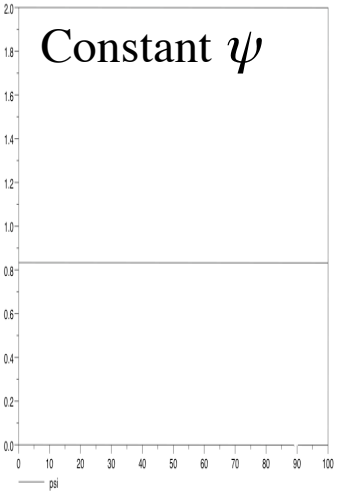
Example: ψ=1(16h)+.5(8h) sq. wave vs. constant (=no) control

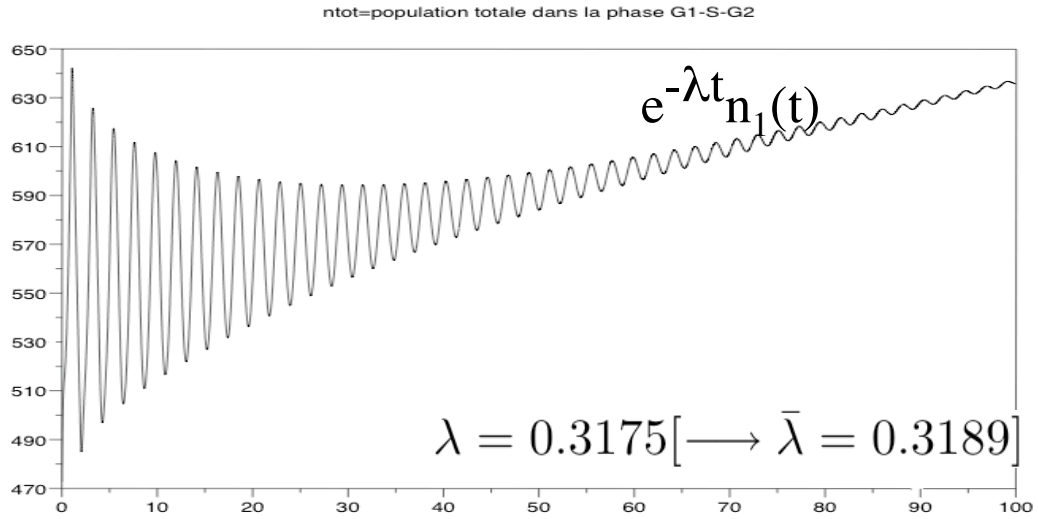
Two phases





Two phases





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

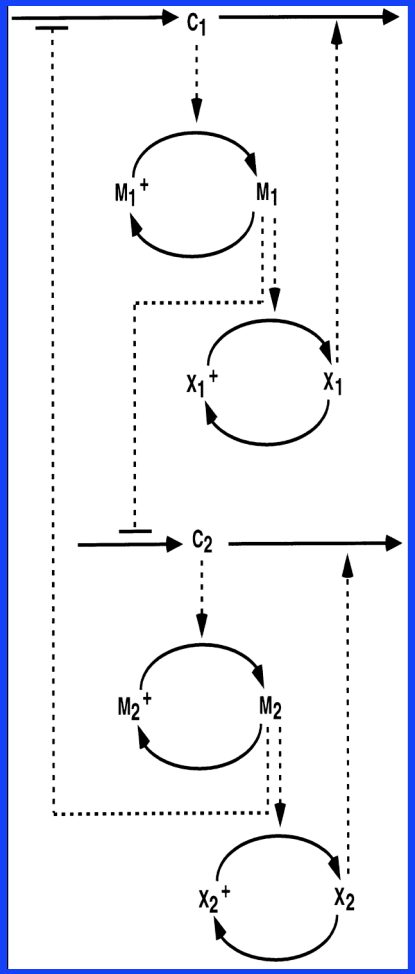
Including more phase transitions in cell cycle control?

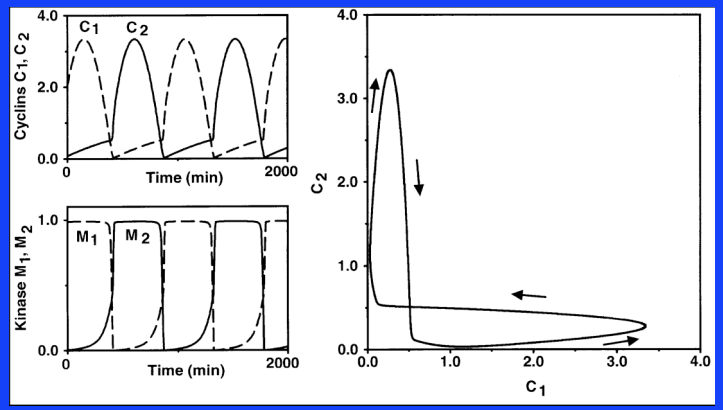
Hint: an existing model for G_1/S and G_2/M synchronisation

(based on the minimum mitotic oscillator *(C, M, X)* by A. Goldbeter, 1996)

i=1: G_1/S (Cyc E, CDK2)

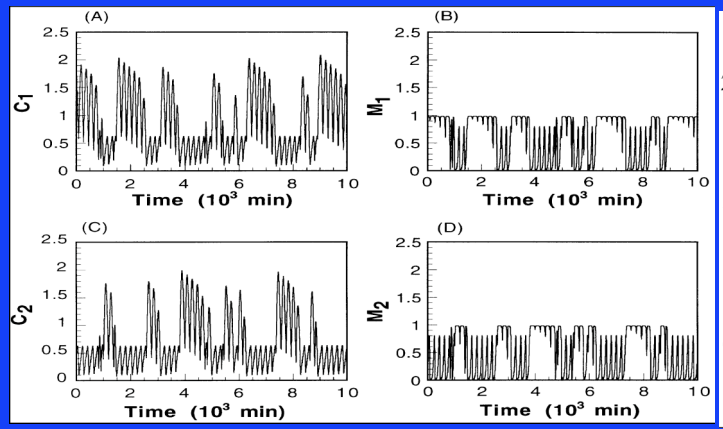
i=2: G_2/M (Cyc B, CDK1)

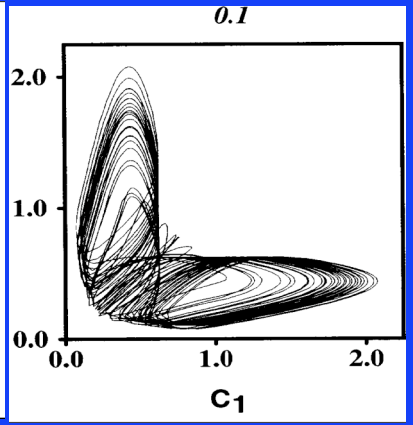




C_i=Cyclin (E, B)
M_i=CDK (2, 1)
X_i=Protease

Changing the coupling strength may lead to:



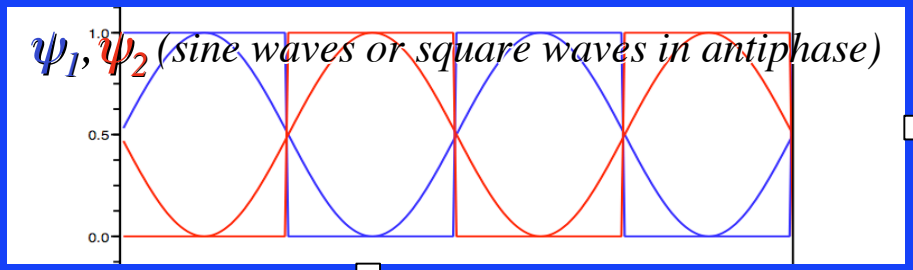


Romond, Gonze, Rustici, Goldbeter, Ann NYAS, 1999

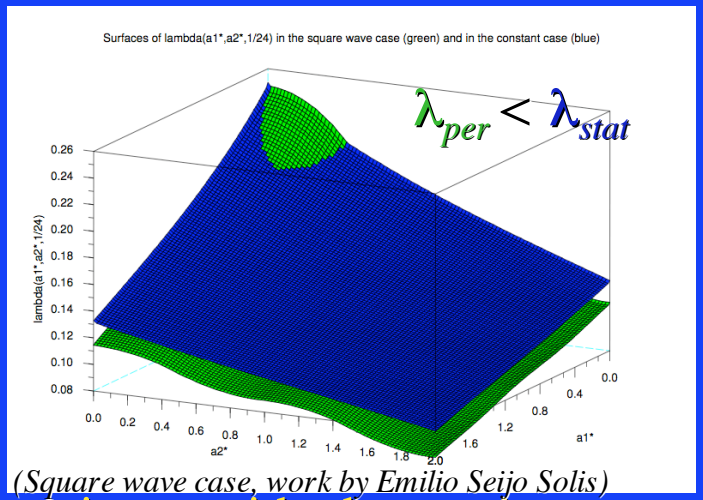
3 phase-model: numerical results with phase-opposed periodic control functions ψ_1 and ψ_2 transitions G_1/S and G_2/M

Numerical simulations have shown that if transition control functions ψ_1 on G_1/S and ψ_2 on G_2/M 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 always lower than the corresponding value λ_{stat} for $\psi_1 = \psi_2 = 0.5$, whatever the durations a_1, a_2 of the first 2 phases (the third one, M, being fixed as 1 h in a total of 24 h for the whole cell cycle, with no control on M/G_1, i.e., $\psi_3 = 1$).

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



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



(Square wave case, work by Emilio Seijo Solis)

…more consistent with observations, assuming

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

(jet-lag=desynchronisation between clocks?)

Nevertheless note also:

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

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

…but only provided that one uses an arithmetico-geometric mean for the $K_{i->i+1}$:

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

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

Clairambault, Gaubert, Perthame C. R. Acad. Sci. Ser. I (Math.) Paris, to appear 2007

…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!)

The circadian system and its disruptions:

representing physiological and disrupted control functions ψ_i

('Circa diem'=approximately one day)

The circadian system…

Central coordination

CNS, hormones, peptides, mediators

Pineal

NPY

NPV

Melatonin

Entrainment by light

RHT

Glutamate

SCN

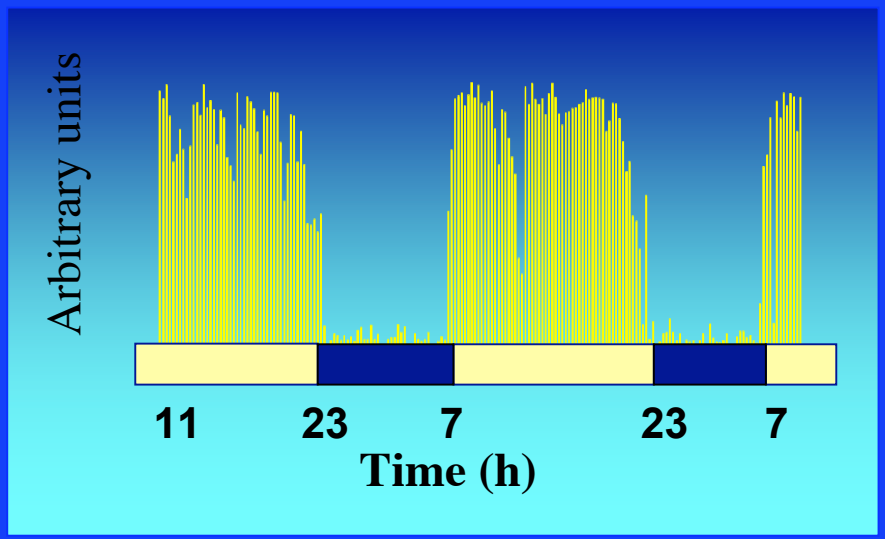
TGFα, EGF

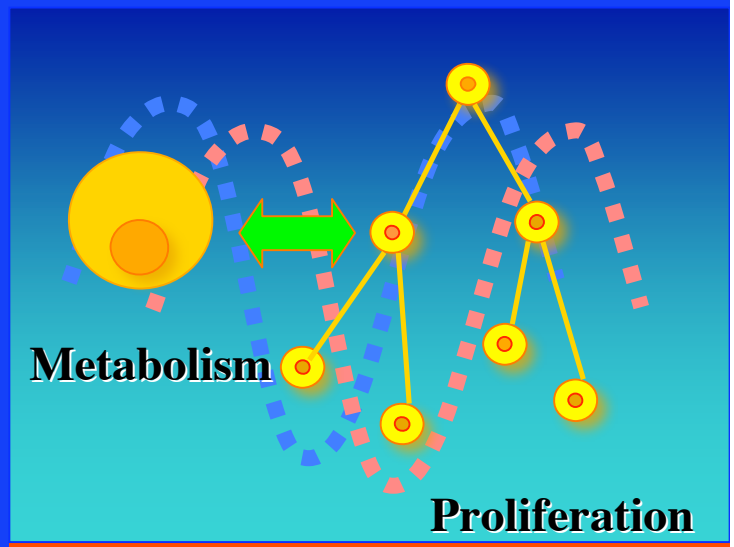
Prokineticin

Glucocorticoids

Food intake rhythm

Autonomic nervous system





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 peripheral cells

SCN=suprachiasmatic nuclei (in the hypothalamus)

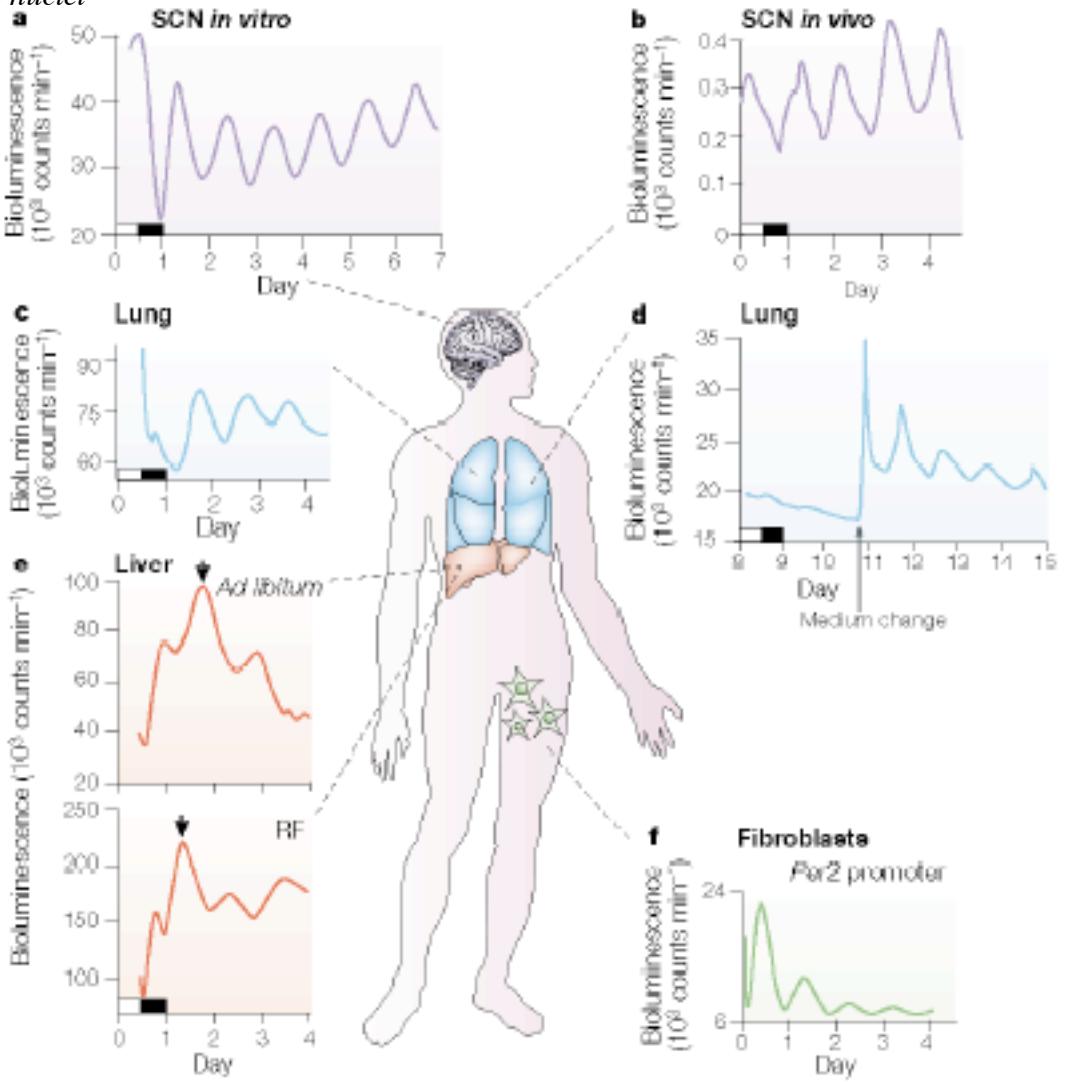
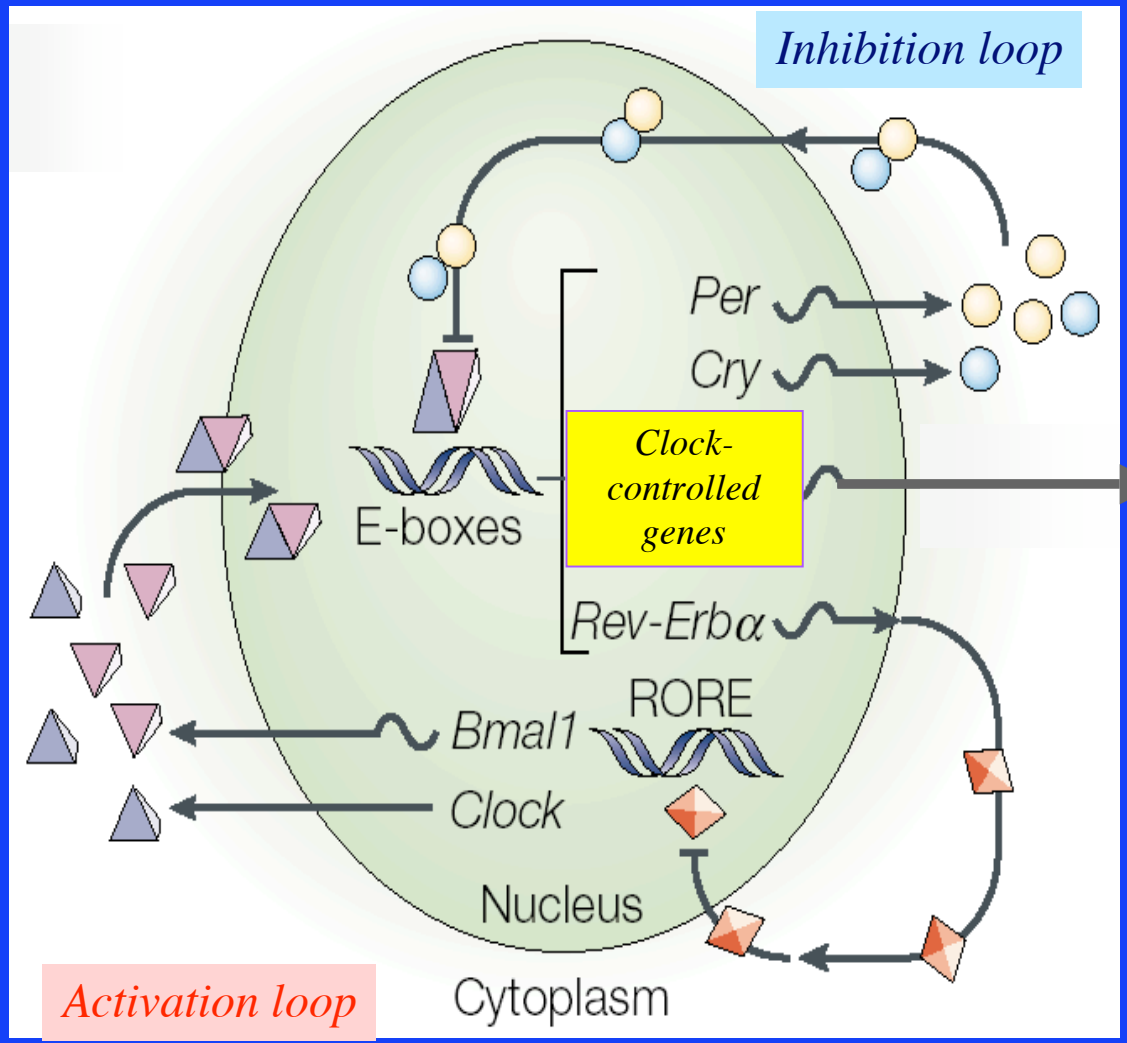


Figure 3 | ***Per::luciferase*** **transgenes reveal a diversity of tissue-based circadian oscillators.**

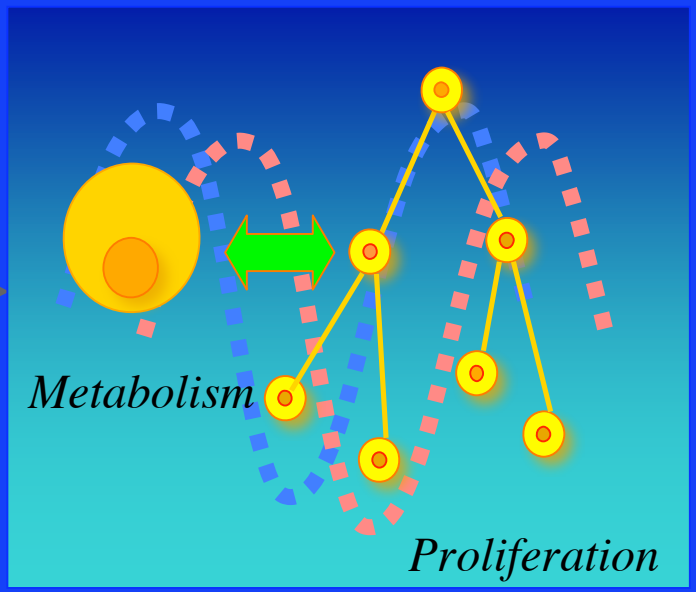
(from Hastings, Nature Rev.Neurosci. 2003)

In each cell: a molecular circadian clock



(after Hastings, Nature Rev. Neurosci. 2003)

Cellular rhythms

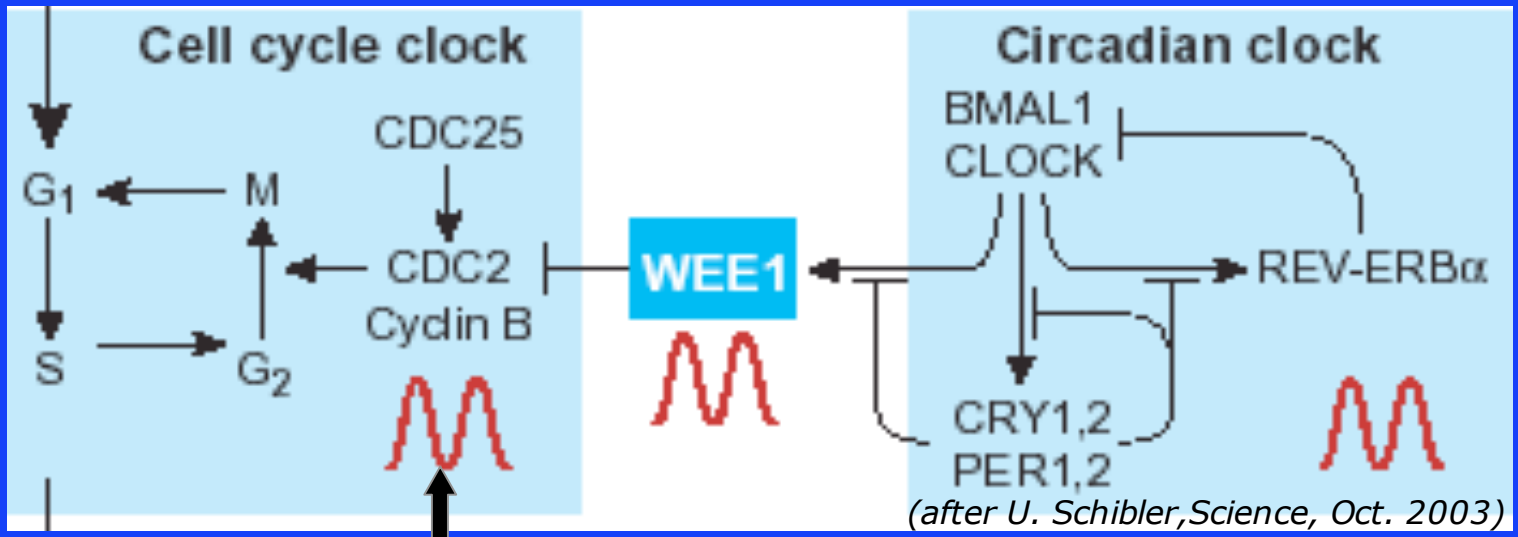


24 h-rhythmic transcription:
10% of genome, among which:
10% : cell cycle
2% : growth factors

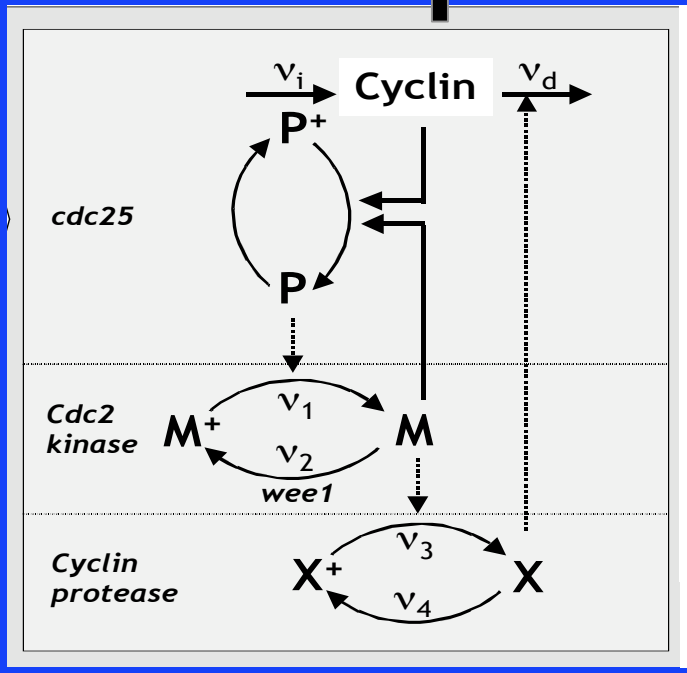
(from Francis Lévi, INSERM U 776 Rythmes Biologiques et Cancers)

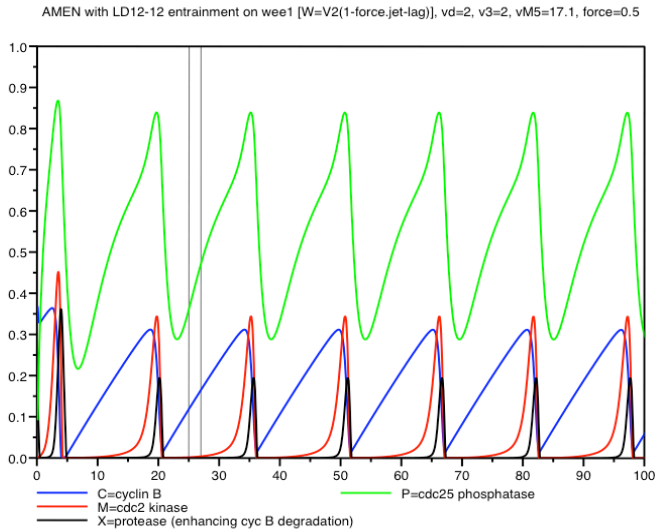
A molecular connection between cell cycle and circadian clock:

cdk1 (cdc2) kinase opens G2/M gate; 24 h-rhythmic Wee1 inactivates cdk1



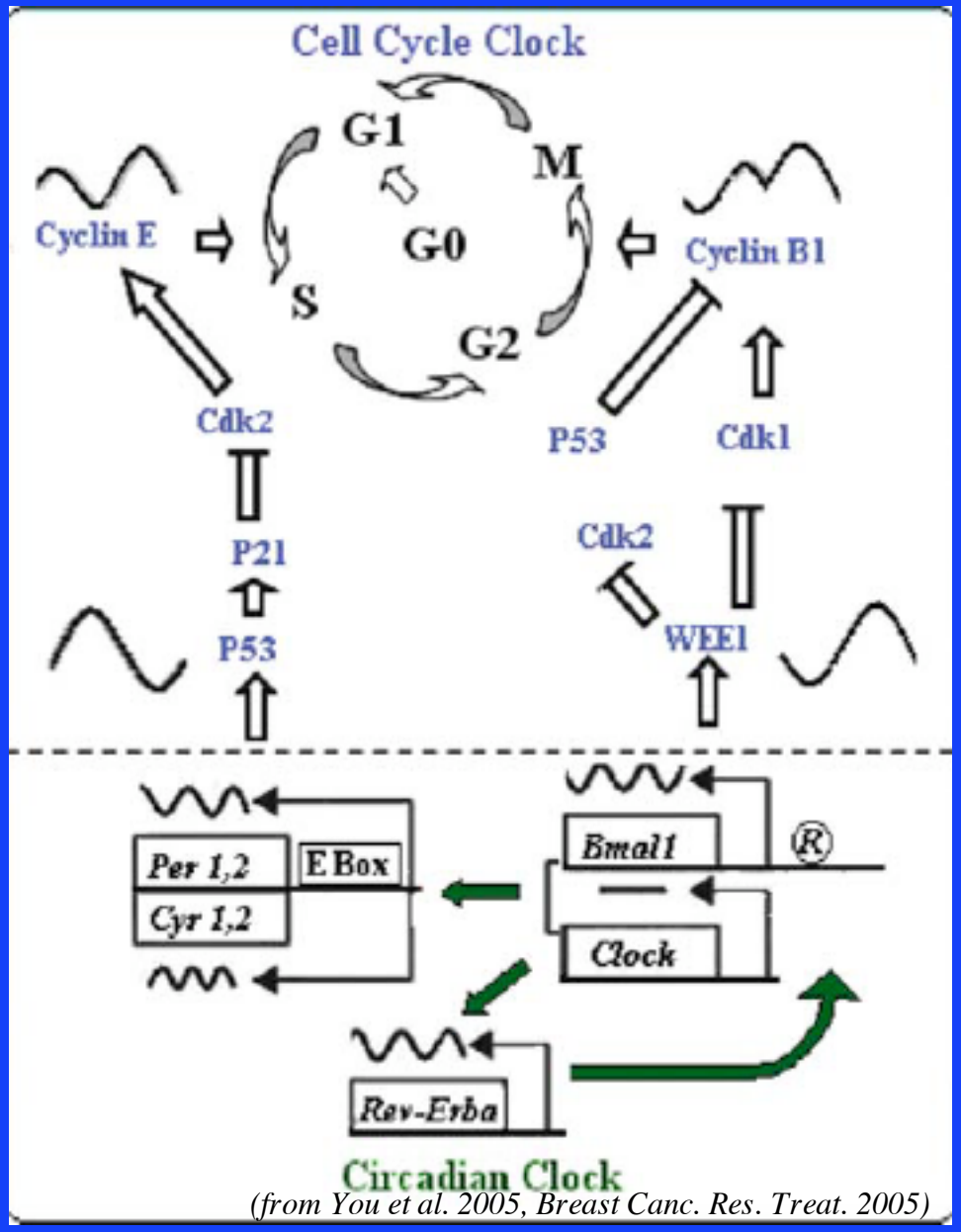
(after U. Schibler,Science, Oct. 2003)





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

More connections between the cell cycle and circadian clocks

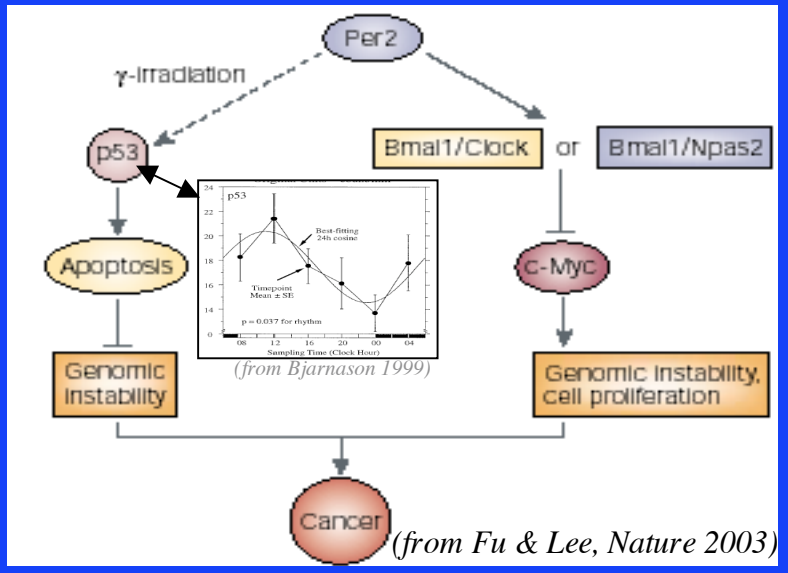


(from You et al. 2005, Breast Canc. Res. Treat. 2005)

1) The circadian clock [Bmal1/Per] might be a synchroniser in each cell between G_1/S and G_2/M transitions *(Wee1 and p21 act in antiphase)*

F. Delaunay, priv. comm. 2007

2) Protein p53, the major sensor of DNA damage, is expressed according to a 24 h rhythm (not altered in Bmal1$^{-/-}$ mice *F. Delaunay*)



(from Bjarnason 1999)

(from Fu & Lee, Nature 2003)

Circadian rhythm disruption in Man

[= loss of synchronisation between circadian molecular clocks?]

- Circadian desynchronisation (loss of rythms of temperature, cortisol, rest-activity cycle) is a factor of poor prognosis in the 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 synchronising factor between phase transitions? And 2) do tumours enhance their development by disrupting the SCN clock?*

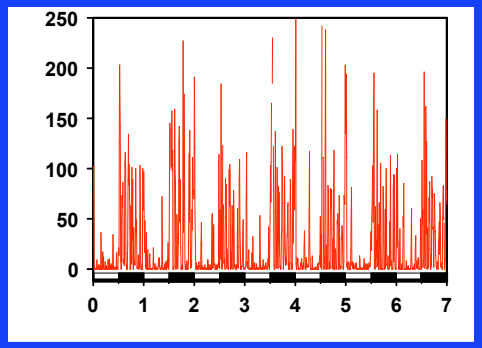
- […and hence resynchronisation therapies (by melatonin, cortisol) in oncology??]

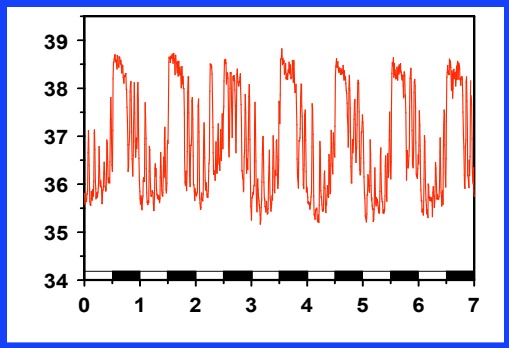
Circadian rhythm disruption by SCN perturbations in mice

Rest-activity

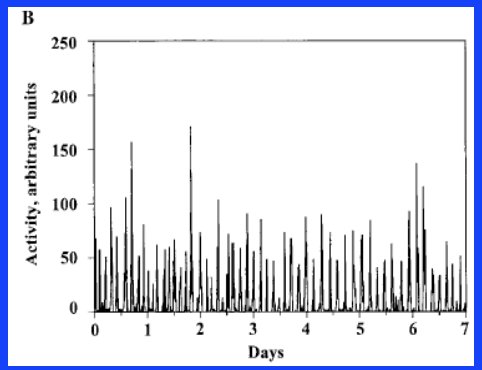
Body temperature

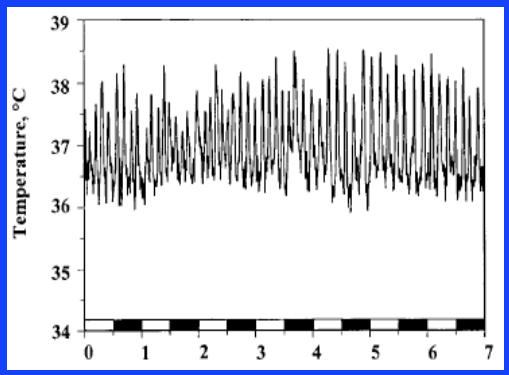
Intact SCN



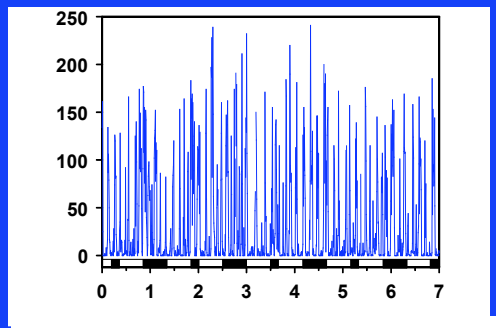


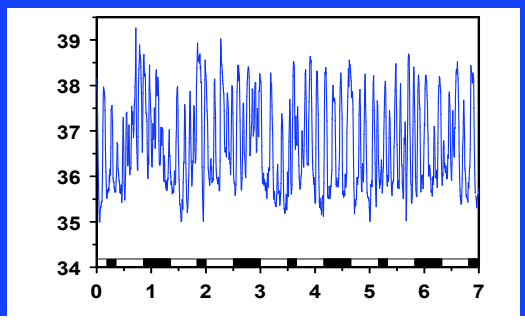
Electrocoagulation





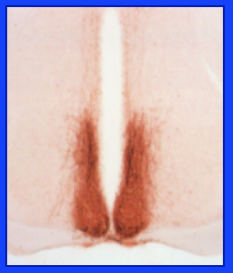
Intact+Jet-lag

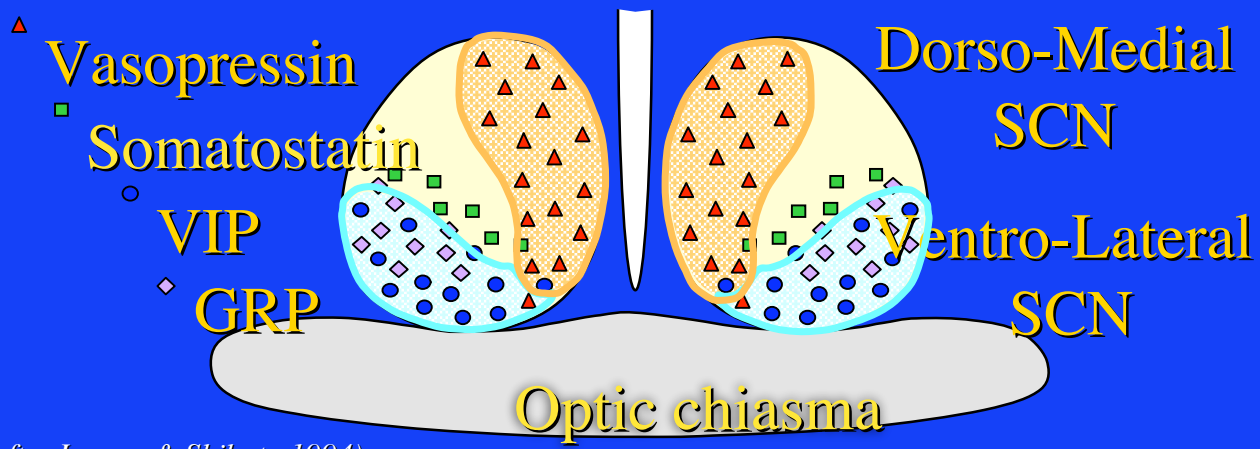




Filipski JNCI 2002, Canc. Res. 2004, JNCI 2005, Canc. Causes Control 2006

The central circadian pacemaker: the suprachiasmatic (SCN) nuclei



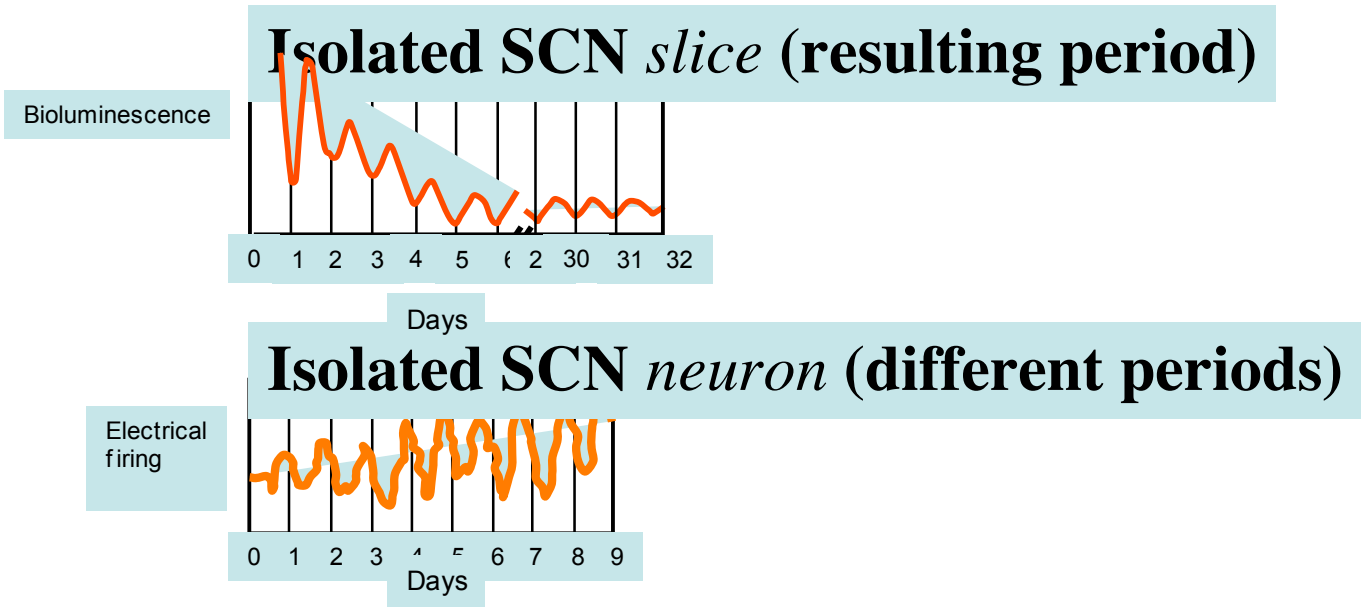


(after Inouye & Shibata 1994)

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

Oscillations in the central pacemaker result from interneuronal coupling and from integration of individual neuronal action potentials



(after Welsh 1995 and Yamazaki 2000)

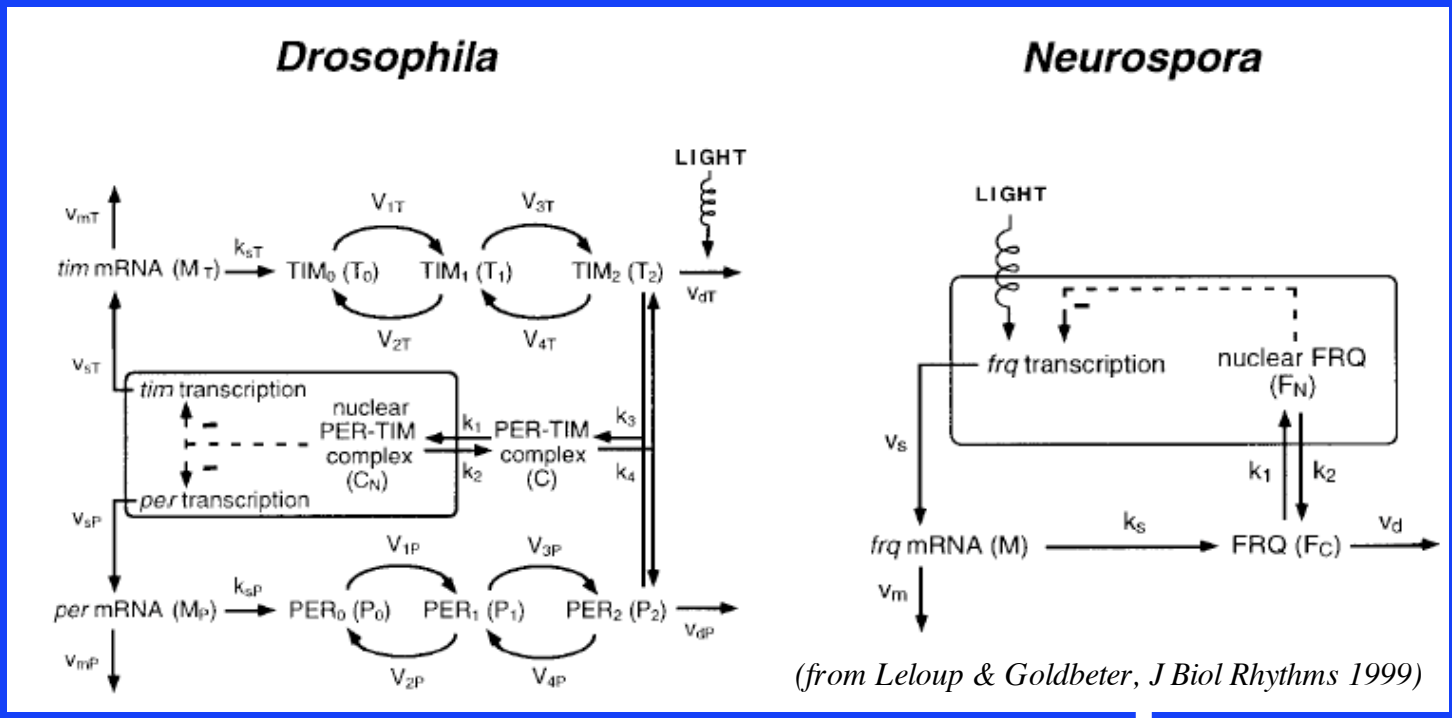
Light entrains the SCN pacemaker but is not mandatory for its rhythmic firing

ODE models of the circadian clock

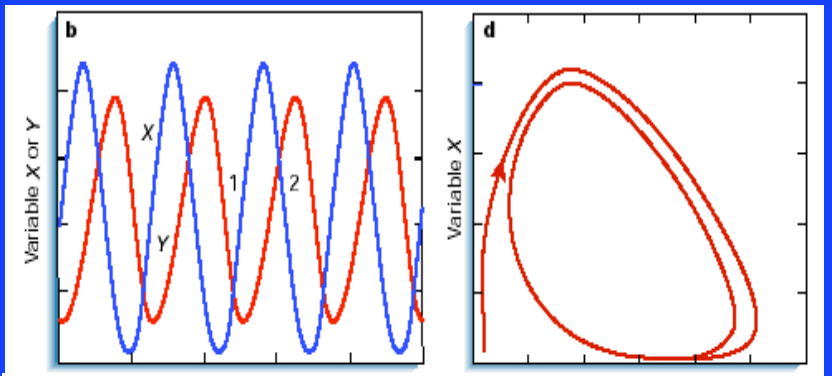
- Goodwin (1965): 3 variables, enzymatic reactions, one sharp nonlinearity
- Forger & Kronauer (2002): Van der Pol-like model, 2 variables
- [Leloup &] Goldbeter (1995, 1999, 2003): 3 (Neurospora *FRQ*); 5 (Drosophila *PER*); 10 (Drosophila *PER+TIM*); 19 (Mammal) variables
- Synchronisation of individual clocks in the SCN: Kunz & Achermann (2003); Gonze, Bernard, Herzel (2005)

All these models show (robust) limit cycle oscillations

Simple mathematical models of the circadian clock



(from Leloup & Goldbeter, J Biol Rhythms 1999)



Limit cycle oscillations (Goldbeter, Nature 2002)

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_m + M}$$

$$\frac{dF_C}{dt} = k_s M - v_d \frac{F_C}{K_d + F_C} - k_1 F_C + k_2 F_N$$

$$\frac{dF_N}{dt} = k_1 F_C - k_2 F_N.$$

Modelling the SCN as a network of coupled oscillators:

diffusive (electric?) coupling between neurons

$$\frac{dmRNA(i)}{dt} = V_s \frac{K^n}{K^n + Z(i)^n} - V_m(i) \frac{mRNA(i)}{K_m + mRNA(i)}$$

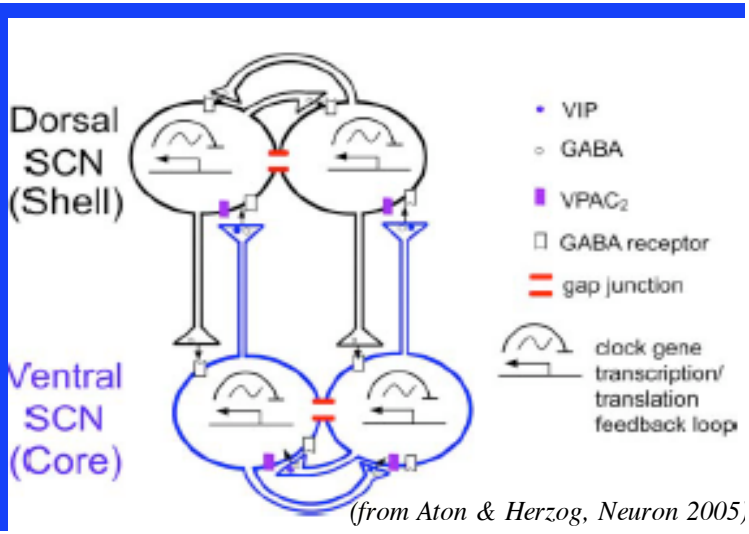
$$\frac{dPER(i)}{dt} = k_s mRNA(i) - V_d \frac{PER(i)}{K_d + PER(i)} - k_1 PER(i) + k_2 Z(i) + K_e \sum_{j \neq i} [PER(j) - PER(i)]$$

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

(after Leloup, Gonze, Goldbeter, J Biol Rhythms 1999)

V_s : $V_s = 1.6\,(1 + L\cos(2\pi t/24))$ target of entrainment by light L; K: target of transcriptional inhibition (e.g. by cytokines); $V_m(i)$: the carrier of variabilility of the oscillatory period in this model

3 variables for the i^{th} neuron that communicates with all other ($j \neq i$) neurons of the SCN through cytosolic PER protein, with coupling constant K_e: electric? gap junctions? VIP / $VPAC_2$ signalling?



(from Aton & Herzog, Neuron 2005)

Pathways from the SCN toward peripheral tissues

(messages suppressed by TTX blockade of interneuronal coupling in the SCN)

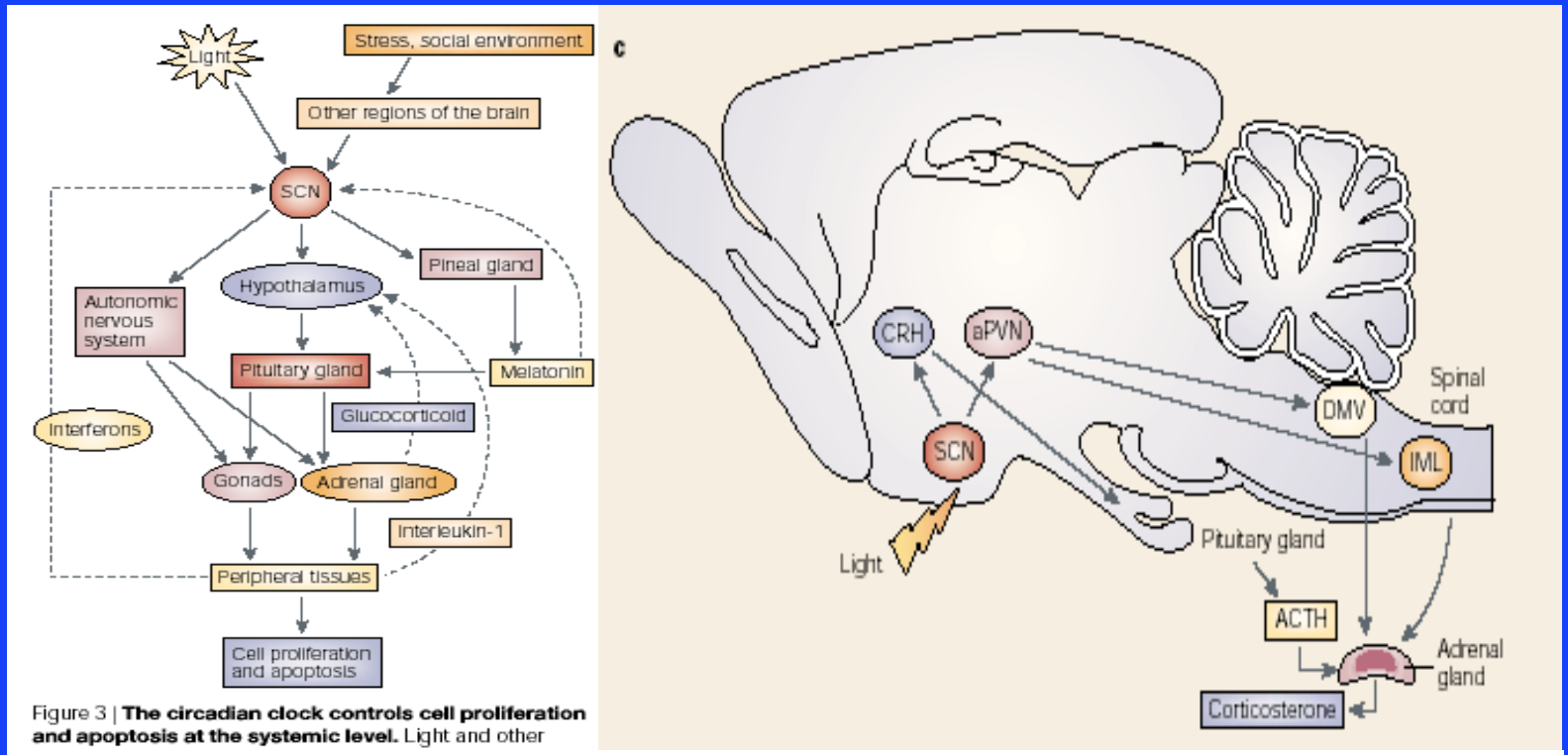


Figure 3 | **The circadian clock controls cell proliferation and apoptosis at the systemic level.** Light and other

(from Fu & Lee, "The circadian clock, pacemaker and tumor suppressor", Nature Rev. Canc. 2003)

Neural messages (ANS), humoral messages (MLT, ACTH) toward periphery
(and secretions: TGFα, prokineticin 2, giving rise to the rest-activity rhythm)

Representation of messages from the SCN to the periphery

$$\frac{dU}{dt} = k_3\overline{PER(NSC)} - k_4U \quad (1)$$

$$\frac{dV}{dt} = k_4U - k_5V \quad (2)$$

$$\frac{dW}{dt} = \frac{aV}{b+V} - cW \quad (3)$$

U = intercentral messenger
V = hormonal messenger (e.g. ACTH)
W= tissue messenger (e.g.. cortisol)

Individual peripheral circadian oscillators:

same model as in the SCN, *without intercellular coupling of clocks*
but with entrainment by a common messenger from the SCN

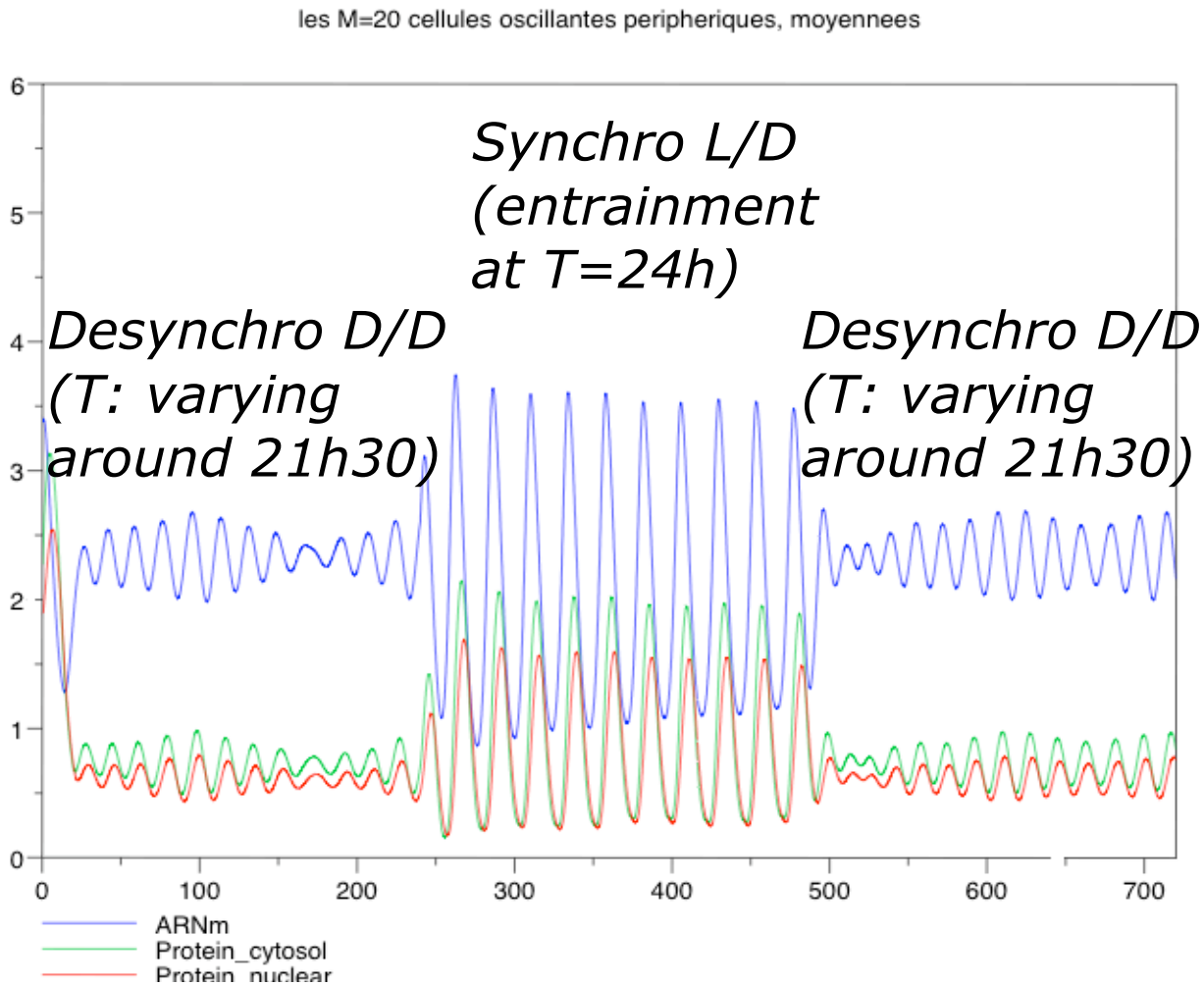
$$\frac{dARN_m}{dt} = V_s \frac{K^n}{K^n + Z^n} - V_m \frac{ARN_m}{K_m + ARN_m}$$

$$Z \longrightarrow Z + r\ W$$

$$(W = \text{messager tissulaire})$$

…determining an average circadian oscillator in each peripheral organ
or tissue, as peripheral clock *PER* averaged over individual clocks

Result = example of disrupted clock: averaged *peripheral* oscillator
1) without *central* entrainment by light; 2) with; 3) without



Resulting Per to control Wee1, that inhibits CDK1= ψ, in proliferating cells

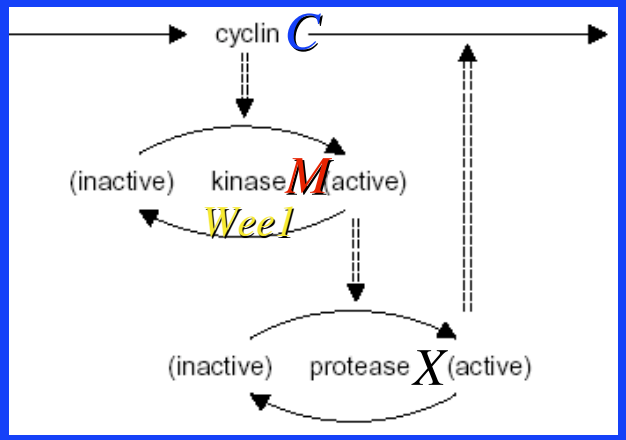
JC, Proc. IEEE-EMBC 2006, IEEE-EMB Mag 2007

Relating circadian clocks to the cell cycle

ODEs to describe progression in the cell cycle at the single-cell level

A. Golbeter's minimal model for the G_2/M transition (the « mitotic oscillator »)

Wee1



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

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

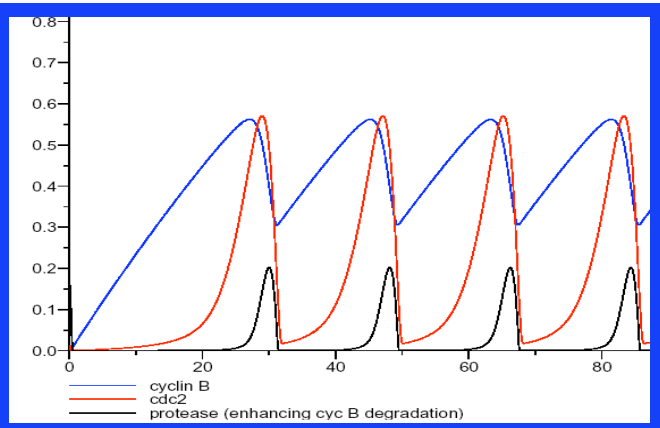
$$\frac{dX}{dt} = v_3 M \frac{(1 - X)}{K_3 + (1 - X)} - V_4 \frac{X}{K_4 + X}.$$

C = cyclin B, M = cyclin dependent kinase cdk1, X = degrading protease

Input: *Per=Wee1*; output: M=Cdk1=ψ

Switch-like dynamics of dimer Cyclin B-cdk1

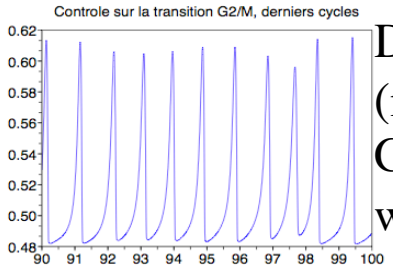
Adapted to describe G_2/M phase transition



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

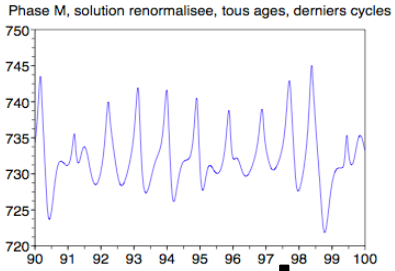
Clock perturbations and cell population proliferation

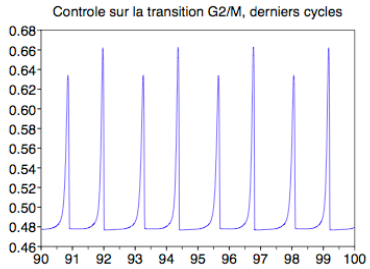
(Wee1 here identified as averaged Per in the circadian clock model)



Desynchronised Wee1 (no entrainment by light): Control cdk1= ψ with perturbed clock

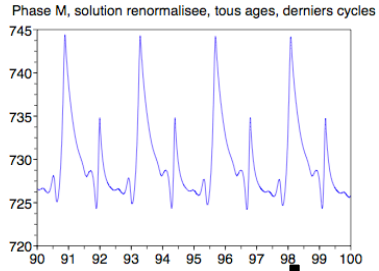
Resulting irregular cell population dynamics in M phase





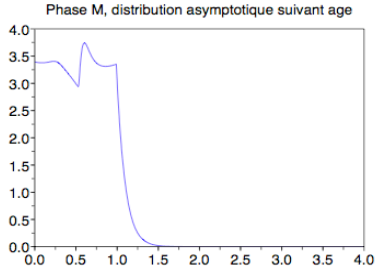
Synchronised Wee1 (entrainment by light): Control cdk1= ψ with unperturbed clock

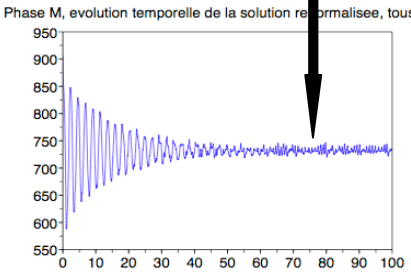
Resulting regular cell population dynamics in M phase



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

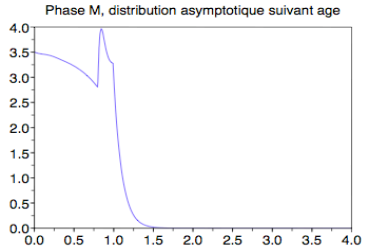
Resulting λ=0.0466

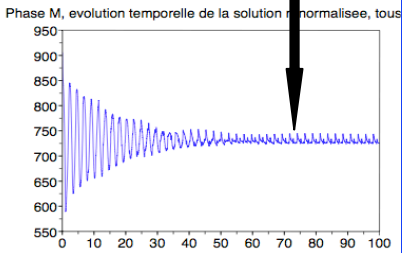




Wee1=Per is synchronised at the central (NSC) level

Resulting λ=0.0452





Molecular PK-PD of anticancer drugs

(representing the effects of drugs on cell cycle control functions ψ_i
with circadian clocks controlling cell detoxification mechanisms)

Treatment of colorectal cancer: by *5FluoroUracil*, *Oxaliplatin* and *Irinotecan*

Pharmacokinetics of IV delivered anticancer drugs:

-binding to plasma proteins, hepatic enzymatic *detoxification*
-distribution from plasma to peripheral tissues
-intracellular shielding (e.g. glutathione *detoxification*) and actual targets
-catabolism (biliary conjugation, renal elimination)

Pharmacodynamics at the tissue level:

1) actions on cell cycle determinants:
-DNA, histones, enzymes (e.g. topoisomerase, thymidylate synthase)
-cyclins and cyclin dependent kinases
-growth factor receptors (EGFRs)
2) other actions: on invasion (ECM degradation), angiogenesis
3) possible limitations due to the occurrence of drug resistances

Drug *Oxaliplatin*:
Modelling molecular PK-PD of oxaliplatin by ODEs: DNA damage, glutathione shielding and DNA repair

$$\frac{dP}{dt} = -[\xi + cl + \lambda L]P + i(t) \tag{1}$$

$$\frac{dL}{dt} = -\lambda PL + \varepsilon\left(N - N_0 - \frac{1}{3}(L - L_0)^3 + r_L(L - L_0)\right) \tag{2}$$

$$\frac{dN}{dt} = -\frac{\omega_L^2}{\varepsilon}(L - L_0) \tag{3}$$

$$\frac{dC}{dt} = -V_{GST}\frac{C(G - G_0)^2}{K_{GST}^2 + (G - G_0)^2} - k_{DNA}CF + \frac{\xi}{2}\frac{P}{\mathcal{W}} \tag{4}$$

$$\frac{dF}{dt} = -k_{DNA}\mathcal{W}CF + k_R F\frac{F_0 - F}{F_0} repair\left(g_R, \theta_1, \theta_2, \frac{F_0 - F}{F_0}\right) \tag{5}$$

$$\frac{dG}{dt} = -V_{GST}\frac{\mathcal{W}C(G - G_0)^2}{K_{GST}^2 + (G - G_0)^2} + \delta\left(S - S_0 - \frac{1}{3}(G - G_0)^3 + r_G(G - G_0)\right) \tag{6}$$

$$\frac{dS}{dt} = -\frac{\omega_G^2}{\delta}(G - G_0) \tag{7}$$

Molecular PK of *Oxaliplatin*: plasma compartment

Mass of active oxaliplatin

Constant clearance

Instantaneous infused drug dose (flow)

Oxaliplatin (*P*) binding to plasma proteins (*L*)

$$\frac{dP}{dt} = -\left[\xi + Cl + \lambda \cdot L\right] \cdot P + i(t)$$

Binding rate of oxaliplatin to plasma proteins

Rate of transfer from plasma to peripheral tissue (cellular uptake)

Mass of plasma proteins (albumin or other hepatic proteins)

ε tunes the robustness of GSH oscillations, from harmonic to relaxation-like

r_L tunes the amplitude of the cycle of plasma proteins

$$\frac{dL}{dt} = -\lambda \cdot P \cdot L + \varepsilon\left(N - N_0 - \frac{1}{3}(L - L_0)^3 + r_L(L - L_0) \right)$$

Hepatic synthesis activity of plasma proteins

ω_L tunes the period of the cycle of plasma proteins

Plasma protein synthesis shows circadian rhythm

$$\frac{dN}{dt} = -\frac{\omega_L^2}{\varepsilon}(L - L_0)$$

Molecular PK of *Oxaliplatin*: tissue concentration

Tissue concentration in free oxaliplatin (C=[DACHPt])

Degradation of free DNA (F) by oxaliplatin (C)

$$\frac{dC}{dt} = -V_{GST}\frac{C(G-G_0)^2}{K_{GST}^2+(G-G_0)^2} - k_{DNA}CF + \frac{\xi}{2}\frac{P}{\mathcal{W}}$$

GST-mediated binding of reduced glutathione (G) to oxaliplatin (C), i.e., cell shielding by GSH

$\mathcal{W}$ = volume of tissue in which the mass P of free oxaliplatin is infused

"Competition" between free DNA (F) and reduced glutathione GSH (G) to bind oxaliplatin (C) in proliferating cells

Molecular PD of *Oxaliplatin* activity in tissue

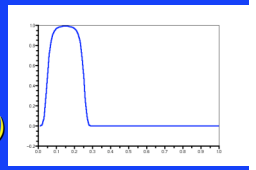
Mass of free DNA

Oxaliplatin (C) on free DNA (F) and glutathione (G) shielding

$$\frac{dF}{dt} = -k_{DNA} \mathcal{W} C F + k_R F \frac{F_0 - F}{F_0} repair\left(g_R, \theta_1, \theta_2, \frac{F_0 - F}{F_0}\right)$$

DNA Mismatch Repair (MMR) function

($\theta_1 < \theta_2$: activation and inactivation thresholds; g_R: stiffness)



Mass of reduced glutathione in target cell compartment

Oxaliplatin cell concentration

δ tunes the robustness of GSH oscillations, from harmonic to relaxation-like

$$\frac{dG}{dt} = -V_{GST} \frac{\mathcal{W} C (G - G_0)^2}{K_{GST}^2 + (G - G_0)^2} + \delta \left(S - S_0 - \frac{1}{3}(G - G_0)^3 + r_G (G - G_0) \right)$$

r_G tunes the amplitude of the cycle of GSH synthesis by GCS = γ-Glu-cysteinyl ligase

Activity of γ-Glu-cysteinyl ligase (GCS)

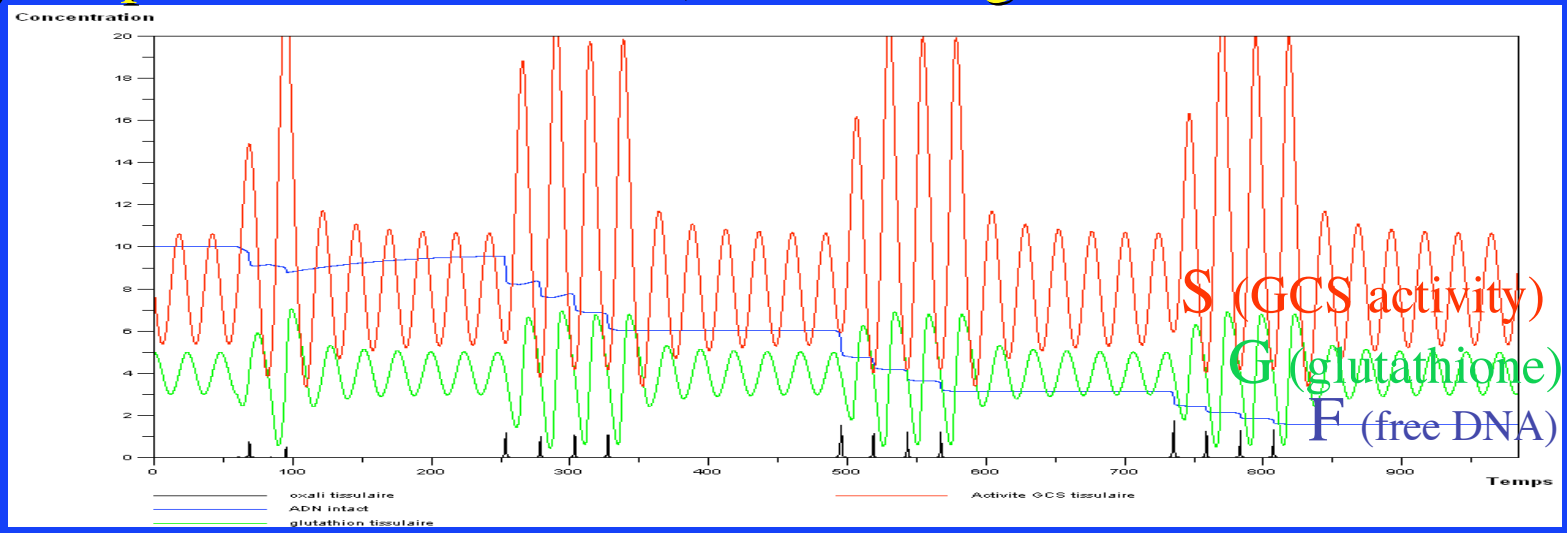
ω_G tunes the period of the cycle of GSH synthesis by GCS

Glutathione synthesis (=detoxification) in cells shows circadian rhythm

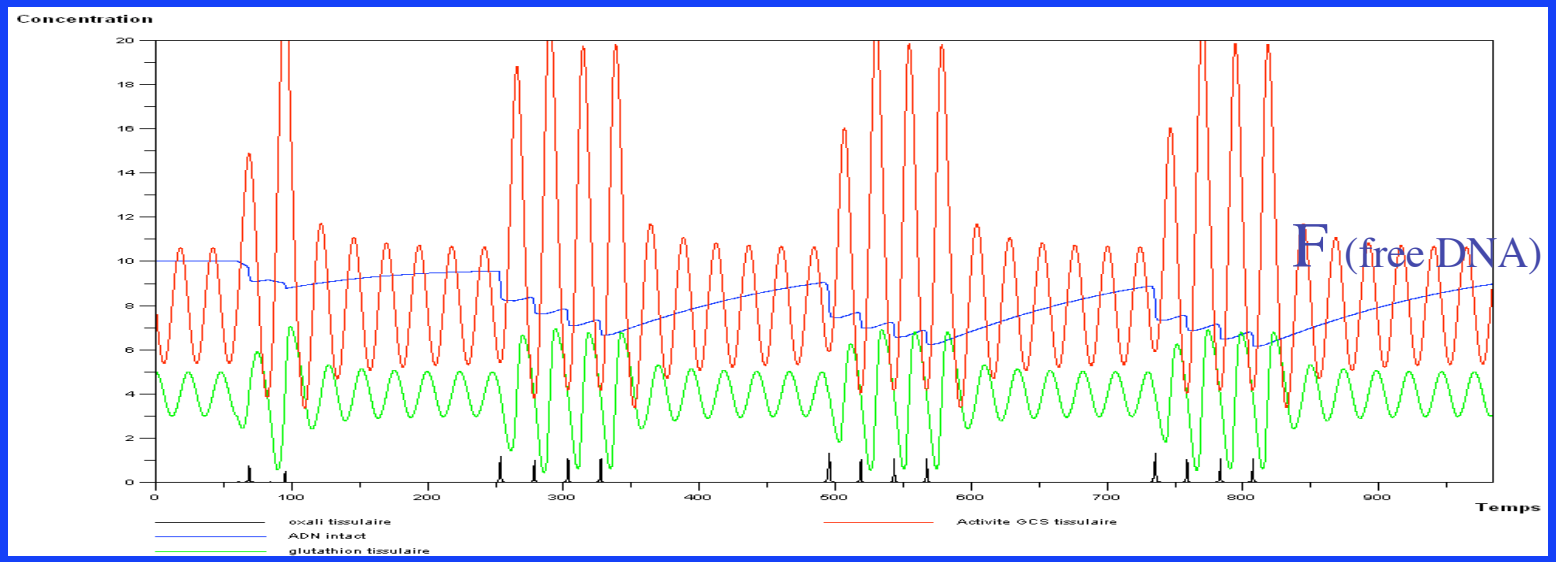
$$\frac{dS}{dt} = -\frac{\omega_G^2}{\delta}(G - G_0)$$

$1\text{-}F/F_0$ =DNA damage

Example: representing the action of *Oxaliplatin* on DNA and ERCC2 polymorphism in tumour cells, to take drug resistance into account:

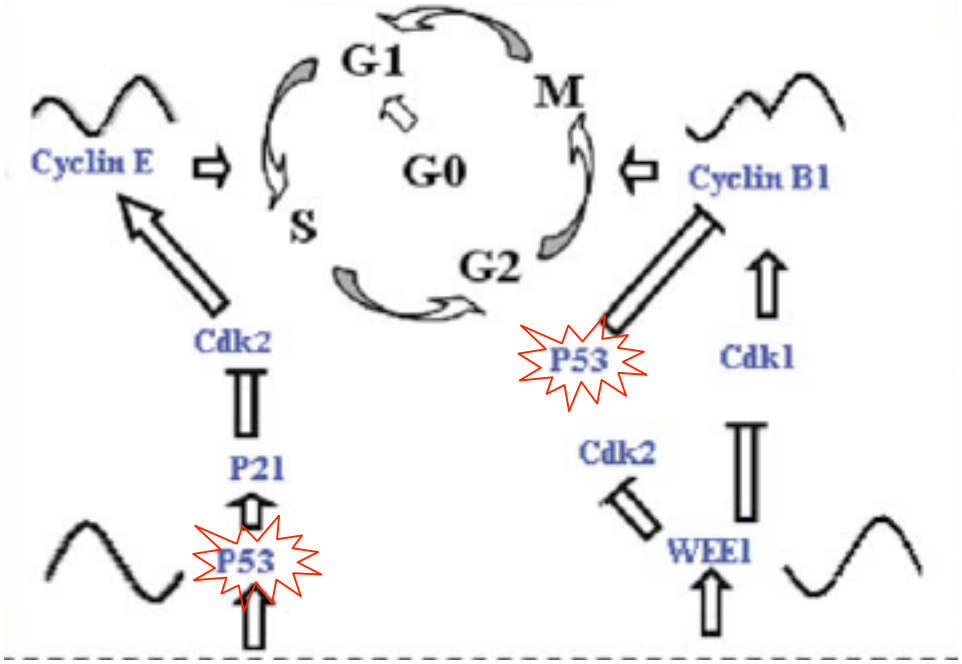


…the same with stronger MMR function (ERCC2=XPD-determined):



(Diminished binding to GSH, diminished cellular uptake, instead of enhanced repair leads to comparable results)

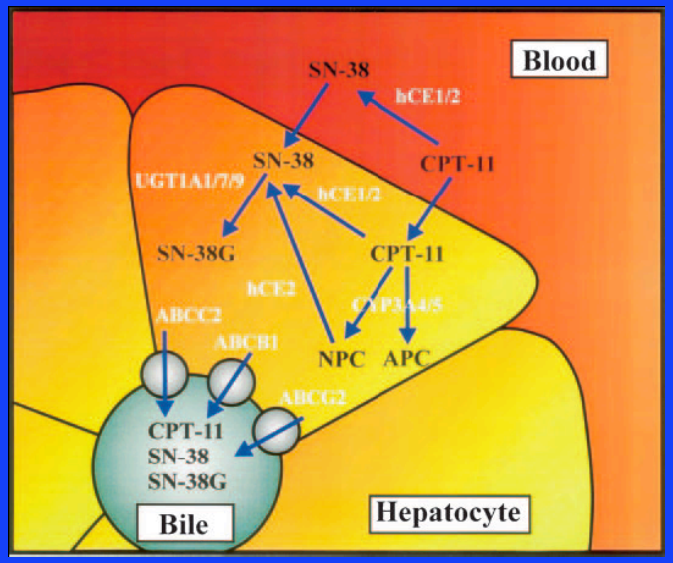
Yet to be studied: p53 to connect DNA damage with cell cycle arrest and apoptosis



Needed: a p53-MDM2 model (existing models by Ciliberto, Chickarmane) to connect DNA damage with cell cycle arrest at checkpoints by ***inhibition of phase transition functions*** ψ_i *and subsequent apoptosis*

(NB: p53 expression is circadian clock-controlled)

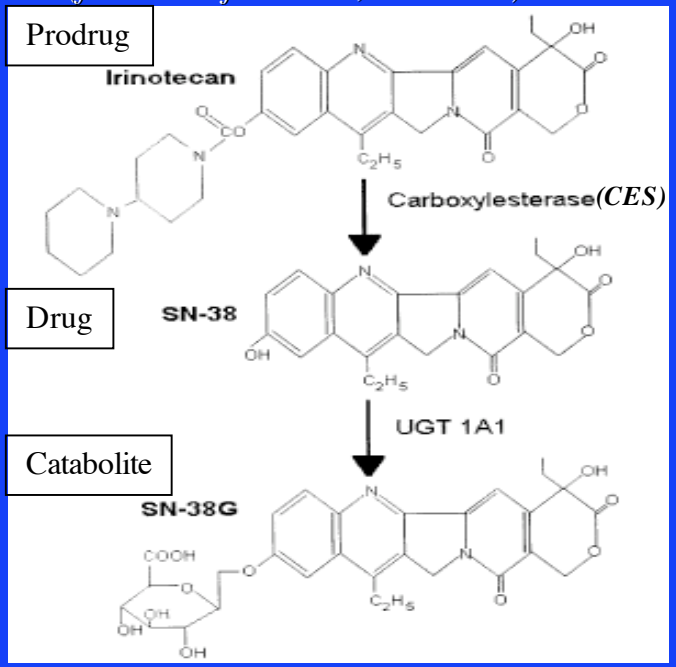
Drug *Irinotecan* (CPT11)



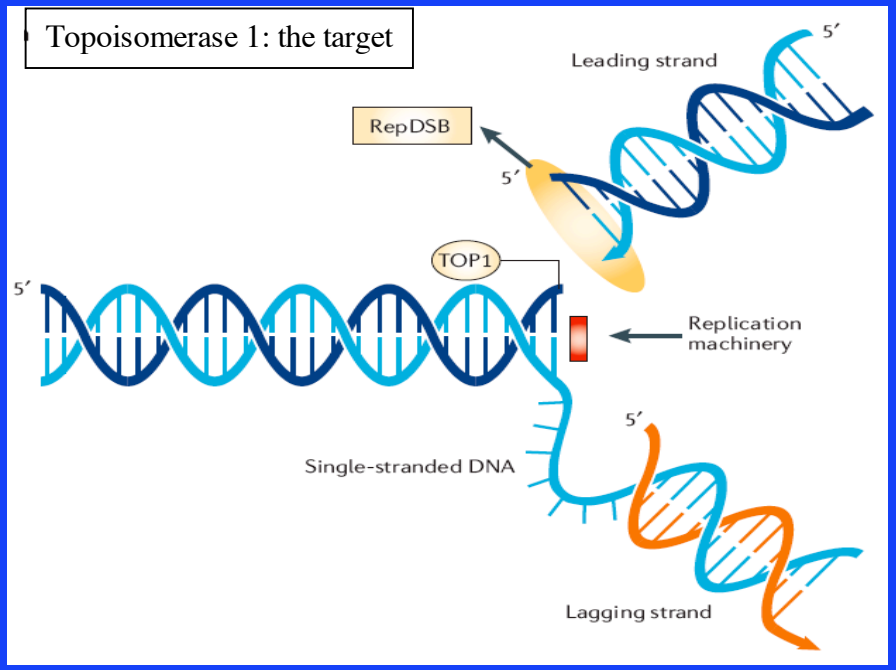
(from Mathijssen et al., JNCI 2004)

Intracellular PK-PD model of CPT11 activity:

- [CPT11], [SN38], [SN38G], [BCGA2 (PGP)], [TOP1], [DNA], [p53], [MDM2]
- Input=CPT11 intracellular concentration
- Output=DNA damage
- Constant activities of enzymes CES and UGT 1A1
- A. Ciliberto's model for p53-MDM2 dynamics



(from Klein et al., Clin Pharmacol Therap 2002)



(from Pommier, Nature Rev Cancer 2006)

Intracellular PK-PD of *Irinotecan* (CPT11)

PK

$$
\begin{cases}
\dfrac{d[CPT11]}{dt} = In(t) - k_1 \dfrac{[CES][CPT11]}{K_{m1} + [CPT11]} - k_{t1} \dfrac{[ABCG2][CPT11]}{K_{t1} + [CPT11]} \\
\dfrac{d[SN38]}{dt} = k_1 \dfrac{[CES][CPT11]}{K_{m1} + [CPT11]} - k_{t2} \dfrac{[ABCG2][SN38]}{K_{t2} + [SN38]} - k_2 \dfrac{[UGT1A1][SN38]^n}{K_{m2}^n + [SN38]^n} \\
\qquad -k_{compl}[SN38][TOP1][ADN_{libre}] + k_{compl_1}[CC] \\
\dfrac{d[SN38G]}{dt} = k_1 \dfrac{[UGT1A1][SN38]^n}{K_{m1}^n + [SN38]^n} - k_{d1}[SN38G] \\
\dfrac{d[ABCG2]}{dt} = k_{t2}[ABCG2] \left(\dfrac{[SN38]}{K_{t2} + [SN38]} + k_{t1} \dfrac{[CPT11]}{K_{t1} + [CPT11]} \right) + -k_{d2}[ABCG2
\end{cases}
$$

PD

$$
\begin{cases}
\dfrac{d[TOP1]}{dt} = k_{top1} \left(1 + \varepsilon \cos \left(\dfrac{2\pi(t - \varphi)}{24} \right) \right) - k_{compl}[SN38][TOP1][ADN_{libre}] + k_{compl_1}[CC] - k_{dtop1}[TOP1] \\
\dfrac{d[DNA_{libre}]}{dt} = -k_{compl}[SN38][TOP1][ADN_{libre}] + k_{compl_1}[CC] + repairDNA([p53_{tot}], [CC_{irr}]) \\
\dfrac{d[CC]}{dt} = k_{compl}[SN38][TOP1][ADN_{libre}] - k_{compl_1}[CC] - k_{irr}[CC] \\
\dfrac{d[CC_{irr}]}{dt} = k_{irr}[CC] - repairDNA([p53_{tot}], [CC_{irr}])
\end{cases}
$$

$$
repairDNA([p53_{tot}], [CC_{irr}]) = -k_{dDNA}[p53_{tot}] \frac{[CC_{irr}]}{J_{DNA} + [CC_{irr}]}
$$

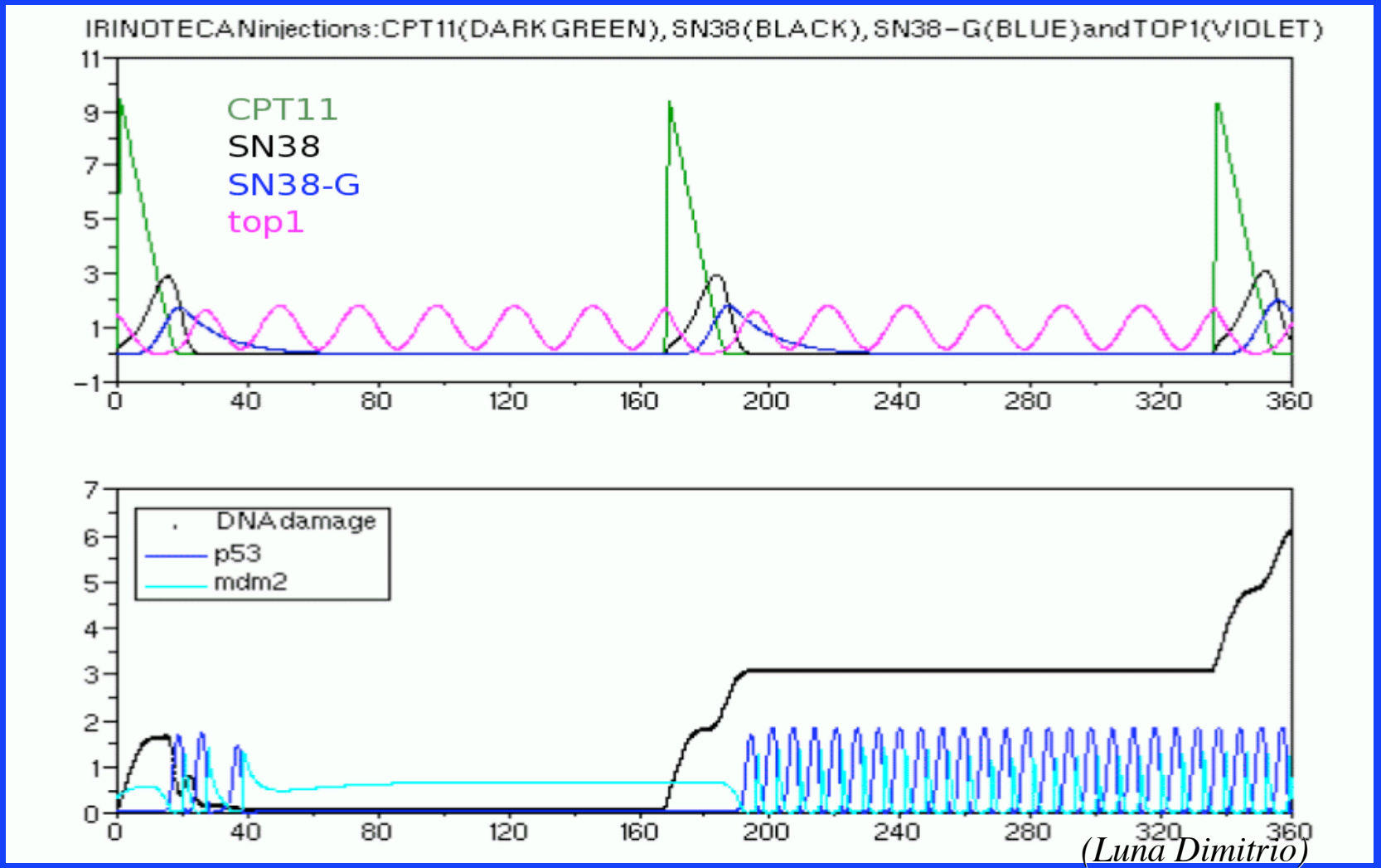
(Luna Dimitrio's Master thesis 2007)

A. Ciliberto's model of p53-mdm2 oscillations

$$
\begin{cases}
\dfrac{d[p53_{tot}]}{dt} &= k_{s53} - k_{d53'}[p53_{tot}] - k_{d53}[p53UU] \\
\dfrac{d[p53U]}{dt} &= k_f[Mdm2_{nuc}][p53] + k_r[p53UU] - [p53U](k_r + k_f[Mdm2_{nuc}]) + -k_{d53'}[p53U] \\
\dfrac{d[p53UU]}{dt} &= k_f[Mdm2_{nuc}][p53U] - [p53UU]k_r - [p53UU](k_{d53'} + k_{d53}) \\
\dfrac{d[Mdm2_{nuc}]}{dt} &= V_{ratio}(k_i[Mdm2P_{cyt}] - k_0[Mdm2_{nuc}]) - k_{bif}[Mdm2_{nuc}] \\
\dfrac{d[Mdm2_{cyt}]}{dt} &= k_{s2'} + \dfrac{k_{s2}[p53_{tot}]^3}{J_s^3 + [p53_{tot}]^3} - k_{d2'}[Mdm2_{cyt}] + k_{deph}[MMdm2P_{cyt}] - \dfrac{k_{ph}}{J_{ph} + [p53_{tot}]}[Mdm2_{cyt}] \\
\dfrac{[Mdm2P_{cyt}]}{dt} &= \dfrac{k_{ph}}{J_{ph} + [p53_{tot}]}[Mdm2_{cyt}] - k_{deph}[Mdm2P_{cyt}] - k_i[MMdm2P_{cyt}] + k_0[Mdm2_{nuc}] - k_{d2'}[MMdm2P_{cyt}]
\end{cases}
$$

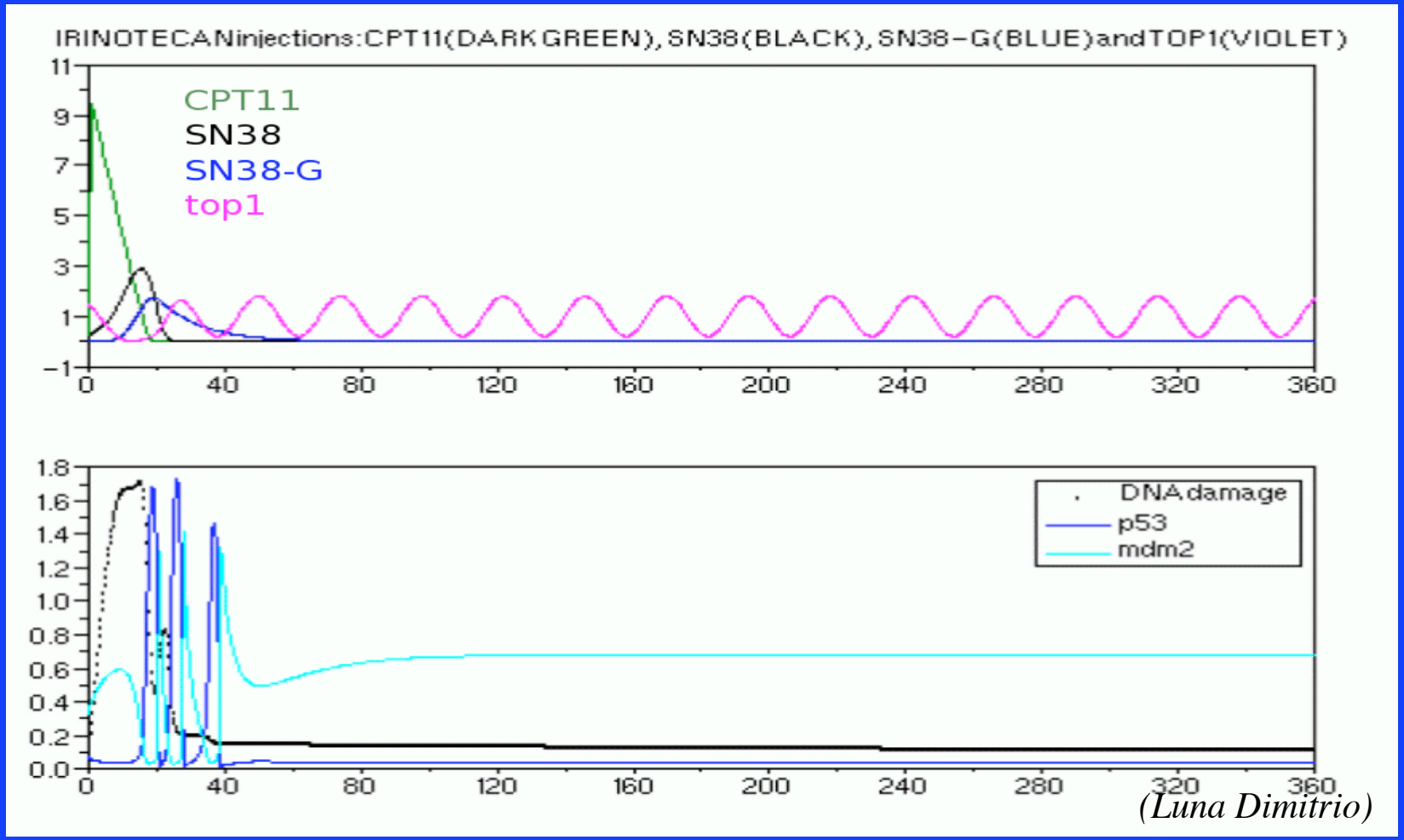
(Ciliberto, Novak, Tyson, Cell Cycle 2005)

PD of *Irinotecan*: p53-MDM2 oscillations can repair DNA damage provided that not too much SN38-TOP1-DNA ternary complex accumulates



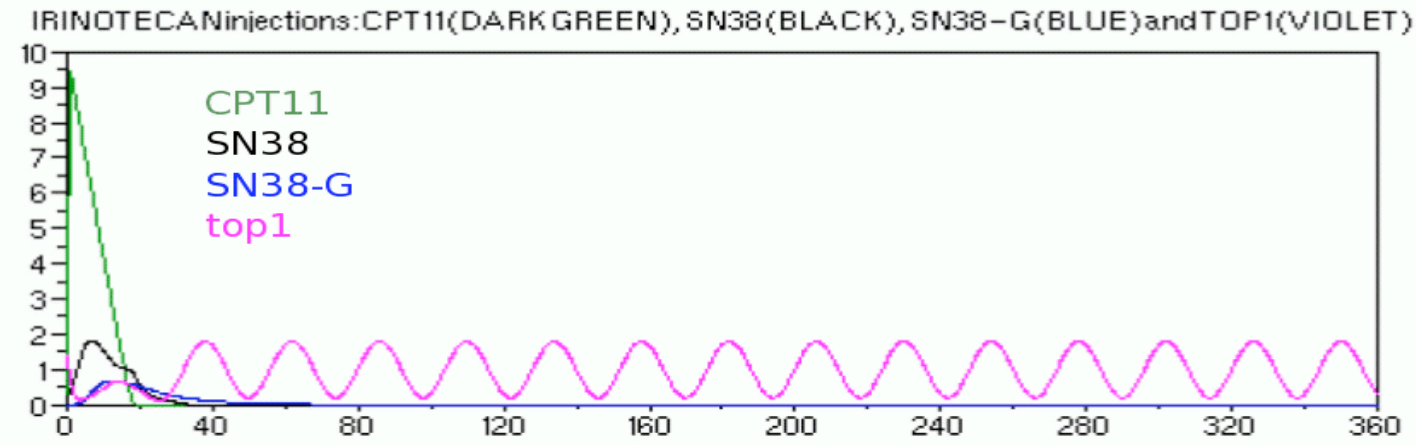
(Intracellular PK-PD of irinotecan and A. Ciliberto's model of p53-MDM2 oscillations)

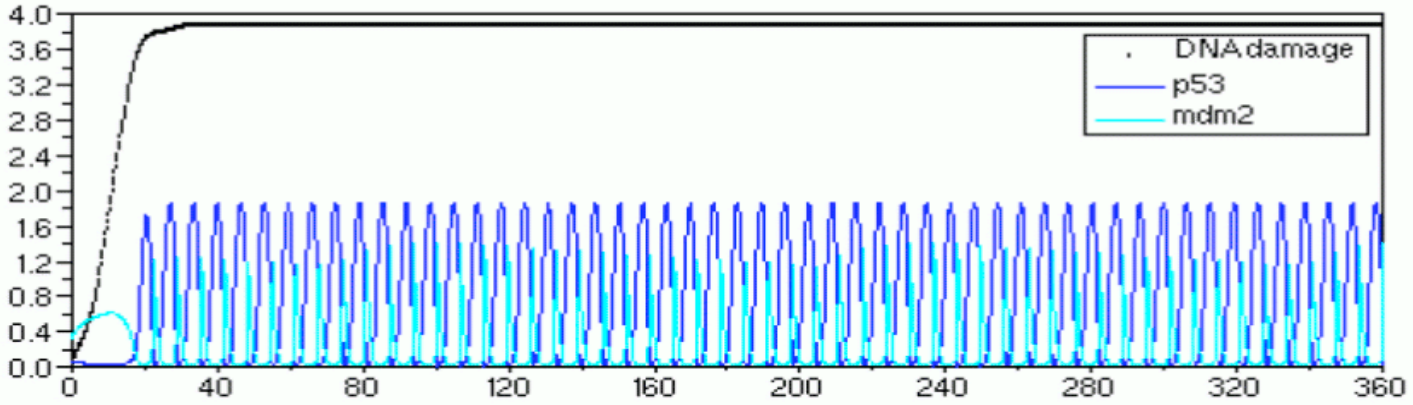
A single infusion of *Irinotecan*, *out of phase* with TOP1 circadian rhythm, creates *reversible damages*: DNA damage is repaired after a few oscillations of p53



(Luna Dimitrio)

A single infusion of *Irinotecan*, *in phase* with TOP1 circadian rhythm, creates *irreversible damages*: p53 oscillations cannot repair the damage to DNA





(Luna Dimitrio)

A rationale for cancer chemotherapy optimisation

Implications of knowledge from physiological cell cycle control modelling for pharmacotherapeutic target designing

1) Theoretical targets of cytotoxic drugs on a cell cycle model should be primarily *the phase transitions, not the apoptosis rates*

2) *Possibly more than one cell cycle transition (G_1/S, G_2/M) must be considered*: the action of drugs should be represented at all checkpoints, with the help of flow cytometry to experimentally measure their cytotoxic properties

3) Non classical cytotoxic drugs such as antagonists of EGFRs (*cetuximab*, *trastuzumab*) or other tyrosine kinase inhibitors (*imatinib*) may require other types of modelling (cell cycle phase independent), with representation of *exchanges between quiescent and proliferative compartments*

4) *Enzymatic systems of drug detoxification* and *active drug efflux transporters* in the liver or peripheral tissues, and their circadian and genetic polymorphism variations, are sources of parameters to be adapted for patient-tailored therapeutics

Ultimate goal: to optimise cancer therapeutics

Use (and restore) the circadian clock to synchronise drug delivery with cell cycle timing, a rationale for cancer chronotherapeutics (aim: to destroy cancer cells while preserving healthy tissues)

Use synergies between drugs with different metabolic mechanisms to enhance their therapeutic effects

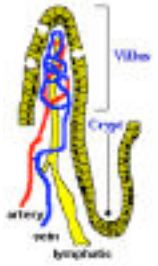
Overcome the occurrence of drug resistance through better understanding of mutations in genes coding for detoxification proteins

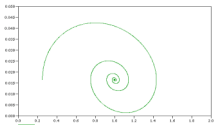
Ultimately: optimal control methods of drug infusion flow delivery…but the objective may be to control a growth exponent λ rather than population numbers

PK-PD simplified model for cancer chronotherapy

Healthy cells (jejunal mucosa)

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



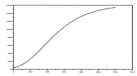


(homeostasis=damped harmonic oscillator)

(PK)

Tumour cells

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



(tumour growth=Gompertz model)

(« chrono-PD »)

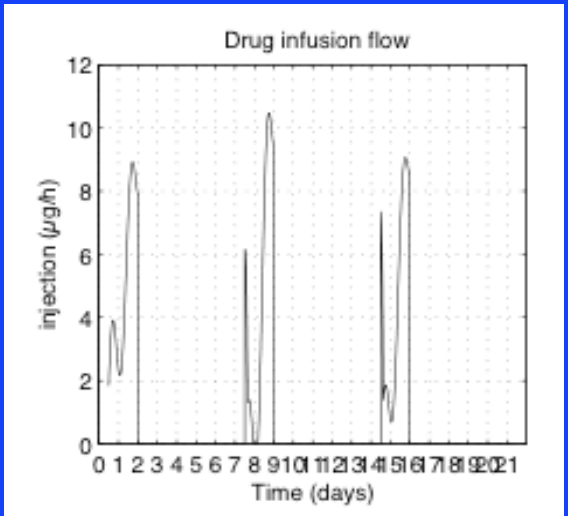
$f(C,t)=F.C^{\gamma}/(C_{50}{}^{\gamma}+C^{\gamma}).\{1+\cos 2\pi(t-\varphi_S)/T\}$

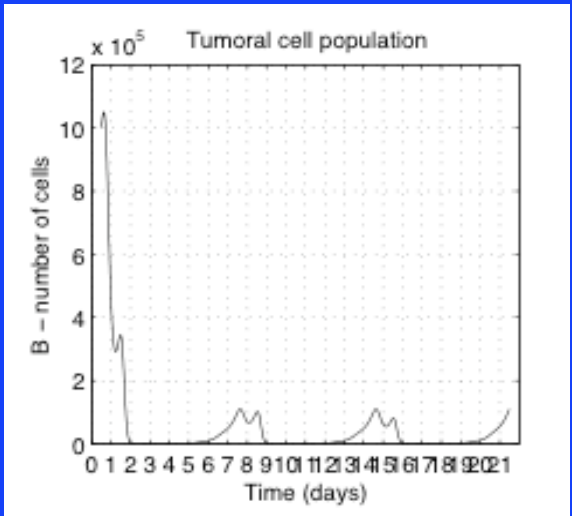
$g(D,t)=H.D^{\gamma}/(D_{50}{}^{\gamma}+D^{\gamma}).\{1+\cos 2\pi(t-\varphi_T)/T\}$

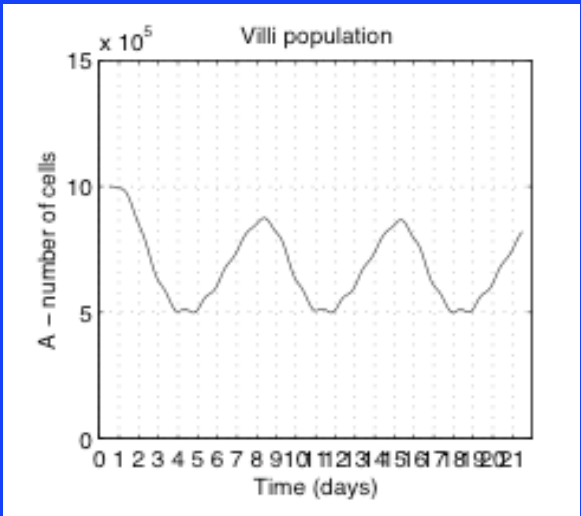
Aim: balancing IV delivered drug anti-tumour efficacy by healthy tissue toxicity

(JC, Pathol-Biol 2003; ADDR 2007)

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







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 adaptable to the patient's state of health

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

(Basdevant, Clairambault, Lévi, M2AN 2005)

Another way to represent healthy and tumour tissue

Age[a]-and-cyclin[x]-structured PDEs with proliferating and quiescent cells for both

(exchanges between *(p)* and *(q)*, 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: total number of cells

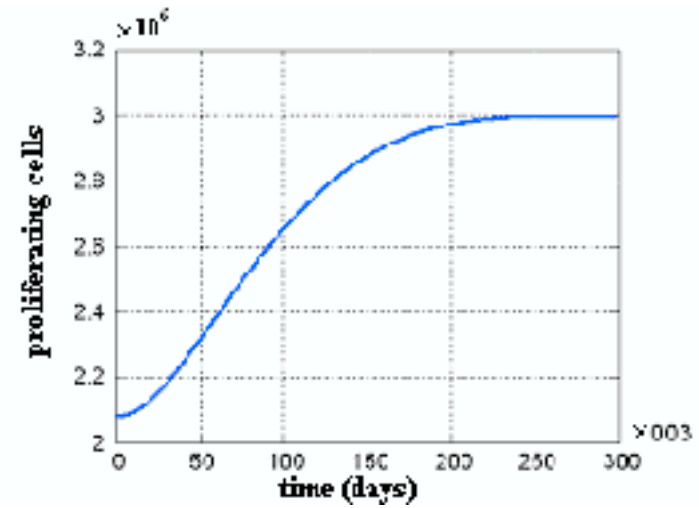
(Here, no circadian control is represented)

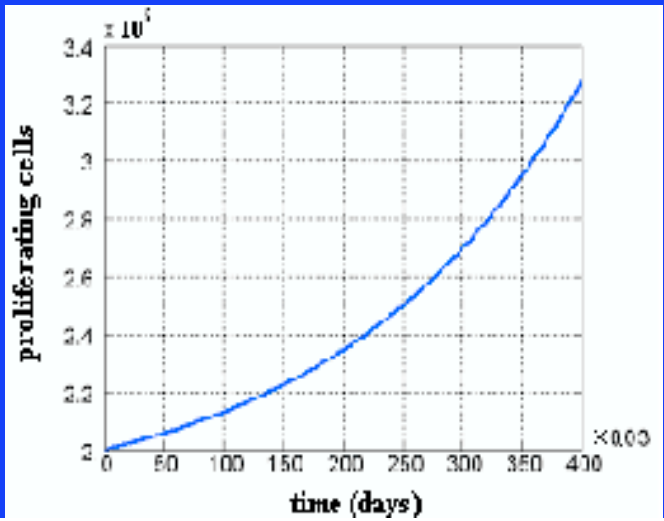
$$G(N) = \frac{\alpha_1\theta^n}{\theta^n + N^n}$$

Healthy tissue recruitment: homeostasis

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

Tumour recruitment: exponential growth





(Bekkal Brikci, Clairambault, Ribba, Perthame submitted 2007; RR INRIA #5941)

Coming next: a 4-day school on cancer modelling in March 2008 near Paris

Models of cancer and its therapeutic control: From molecules to the organism.

CEA-EDF-INRIA Winter school in Rocquencourt (close to Versailles, France).
Targeted dates: March 11-14, 2008. Scientific organisers: J. Clairambault and D. Drasdo.

Tentative programme of lectures (all given in English):

1. *The cell division cycle and its control: individual cells and proliferating cell populations.*
2. *Tissue proliferation and invasion: from individual-based to continuum models.*
3. *Molecular networks: a systems biology approach to robustness and implications for cancer.*
4. *Therapeutic optimisation problems in oncology: side effects, resistance, synergies.*

Proposed 2-hour lectures (3 lectures each of the 4 days of the school):

1. ODE models for the cell cycle / PDE (age or DNA content-structured) models for the cell cycle / Delay Differential Equations for proliferating cell populations.

2. Tissue proliferation and invasion phenomena / From individual-based to continuous models / Probabilistic and deterministic models of tumour growth.

3. Molecular networks, fragility and robustness in cancer / Gene evolutionary dynamics of cancer / Gene evolutionary continuous models (adaptive evolution).

4. Therapeutic optimisation: minimising toxicity by using anticancer drug synergies with chronotherapy (and optimal control) / Therapeutic optimisation: overcoming drug resistances by using drug synergies. / Targeting stem cells.

+ Complementary technical half-an-hour lectures: (1 each day): focus on: Flow cytometry / Cell and tissue image processing / DNA microarray analysis / Cancer databank design.