Cell proliferation, circadian clocks and molecular pharmacokinetics-pharmacodynamics to optimise cancer treatments

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Outline of the lectures

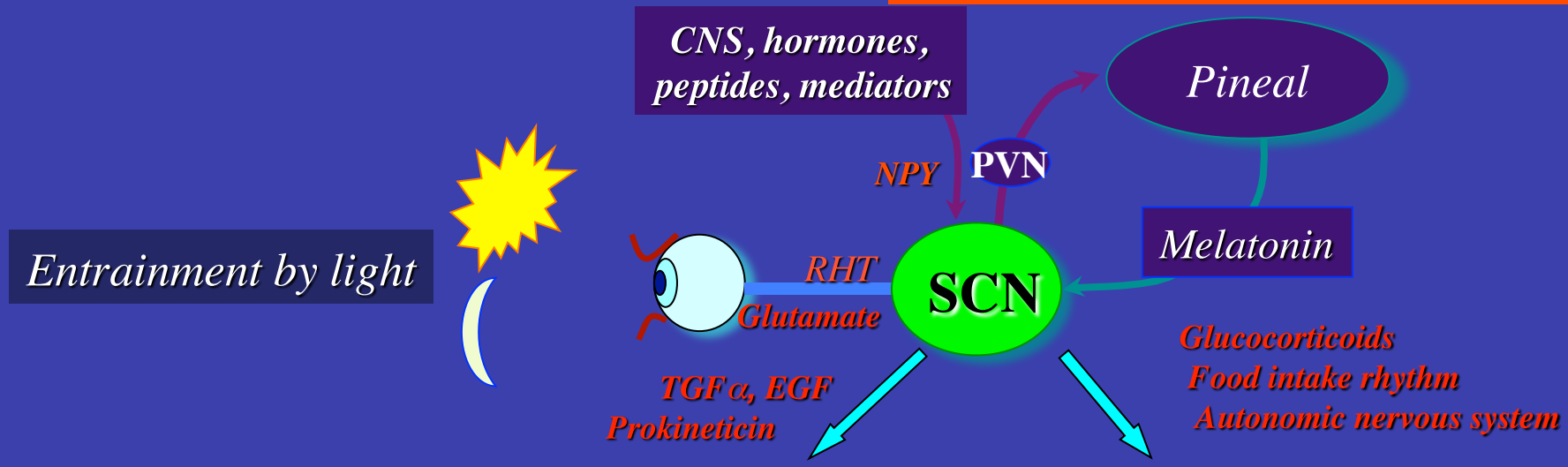
- 0. Introduction and general modelling framework
- 1. Modelling the cell cycle in proliferating cell populations
- 2. Circadian rhythm and cell / tissue proliferation
- 3. Molecular pharmacokinetics-pharmacodynamics (PK-PD)
- 4. Optimising anticancer drug delivery: present and future
- 5. More future prospects and challenges

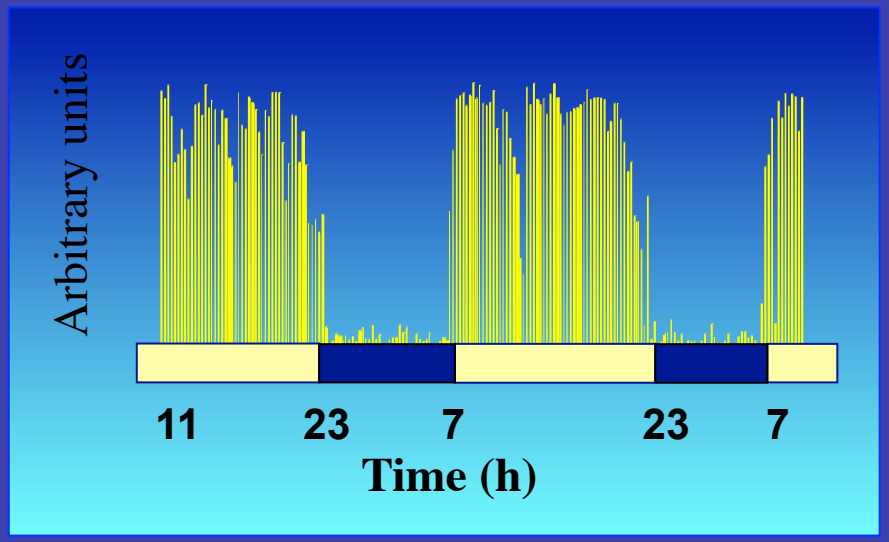
Circadian rhythm and cell / tissue proliferation

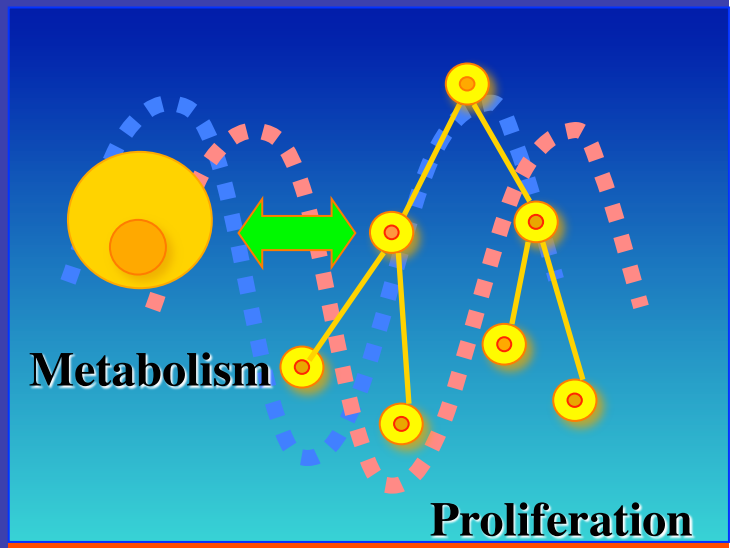
The circadian system (of mice and men)

The circadian system…

Central coordination







Rest-activity cycle: open window on SCN central clock

Peripheral oscillators

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

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

SCN=suprachiasmatic nuclei (hypothalamic)

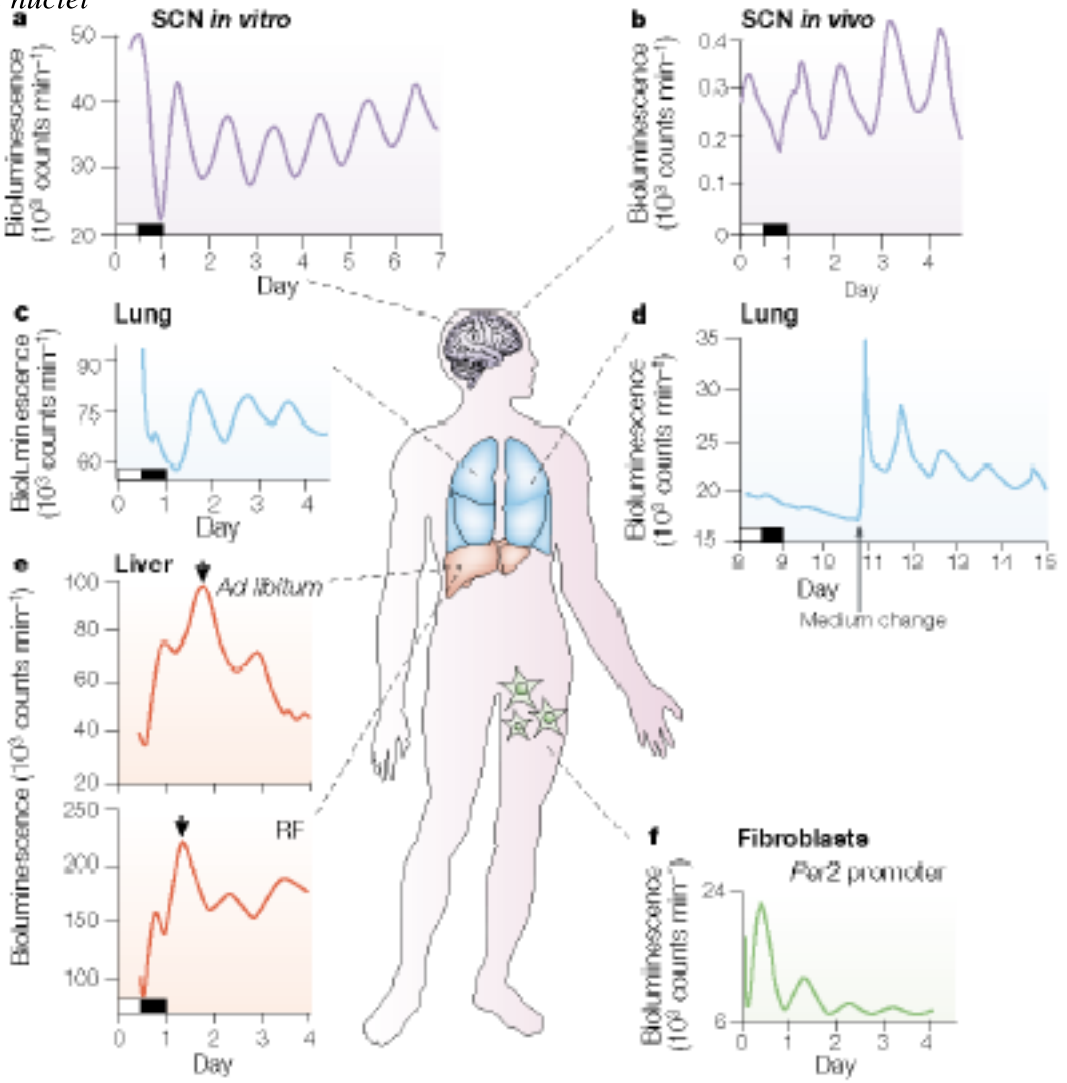
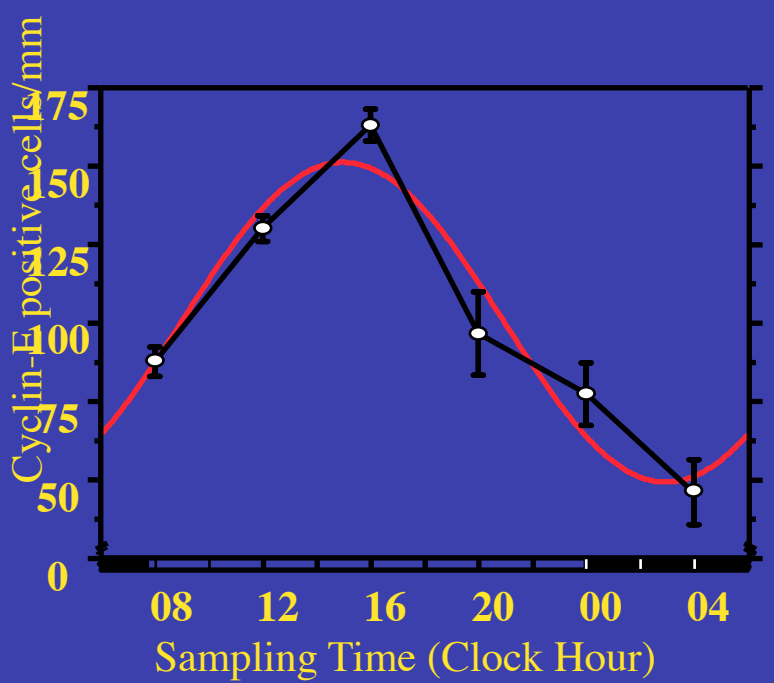


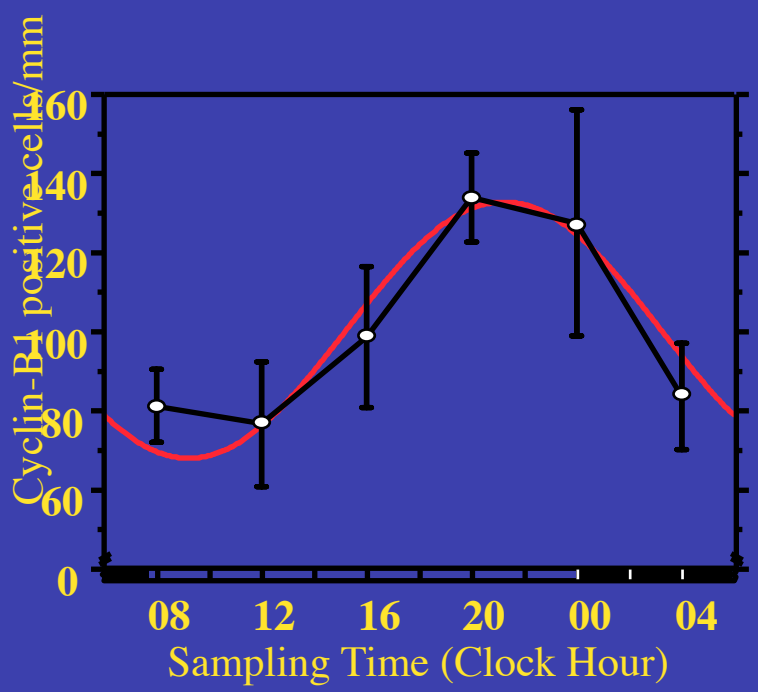
Figure 3 | ***Per::luciferase*** **transgenes reveal a diversity of tissue-based circadian oscillators.**

(from Hastings, Nature Rev.Neurosci. 2003)

Circadian rhythms in the Human cell 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)

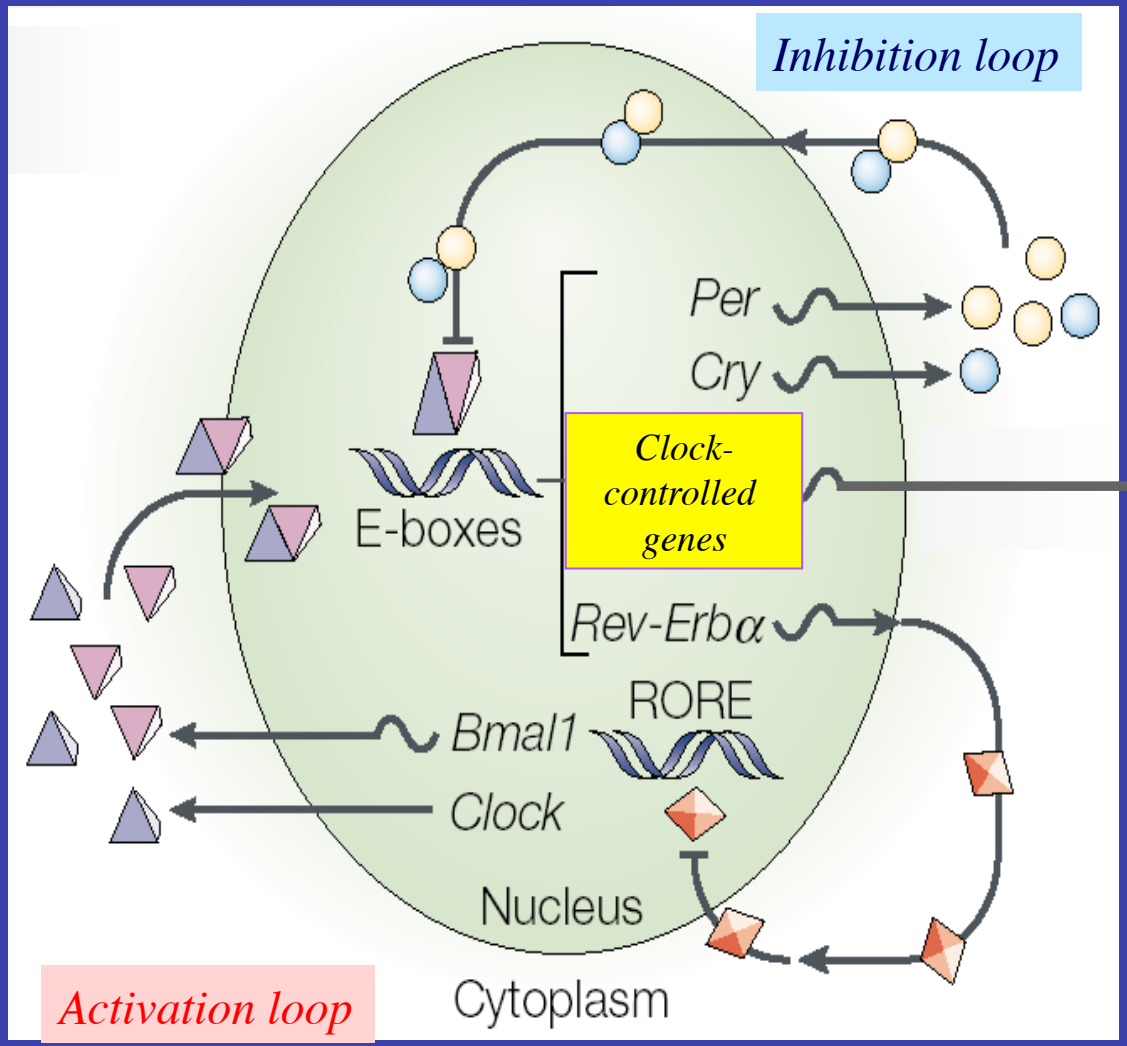




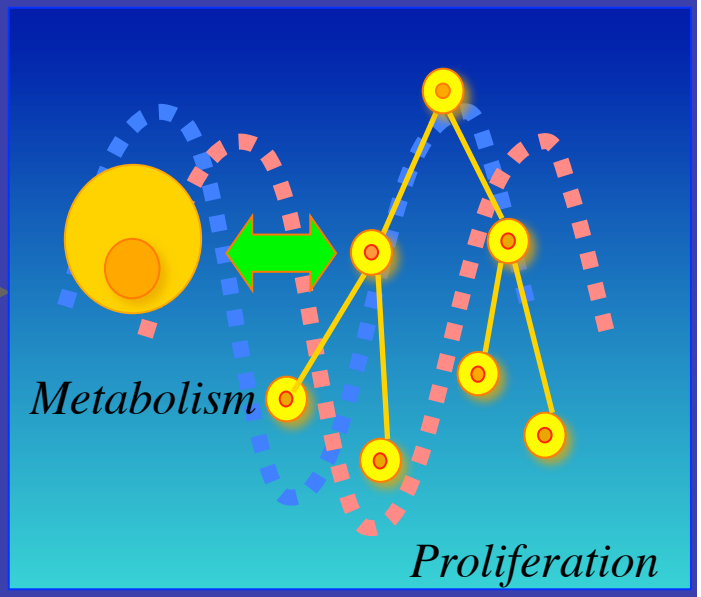
Nuclear staining for Cyclin-E and Cyclin-B1. Percentages of mean ± S.E.M. in oral mucosa samples from 6 male volunteers. Cosinor fitting, $p < 0.001$ and $p = 0.016$, respectively.

(after Bjarnason et al. Am J Pathol 1999)

In each cell: a molecular circadian clock



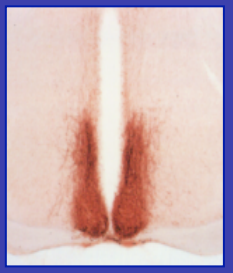
Cellular rhythms

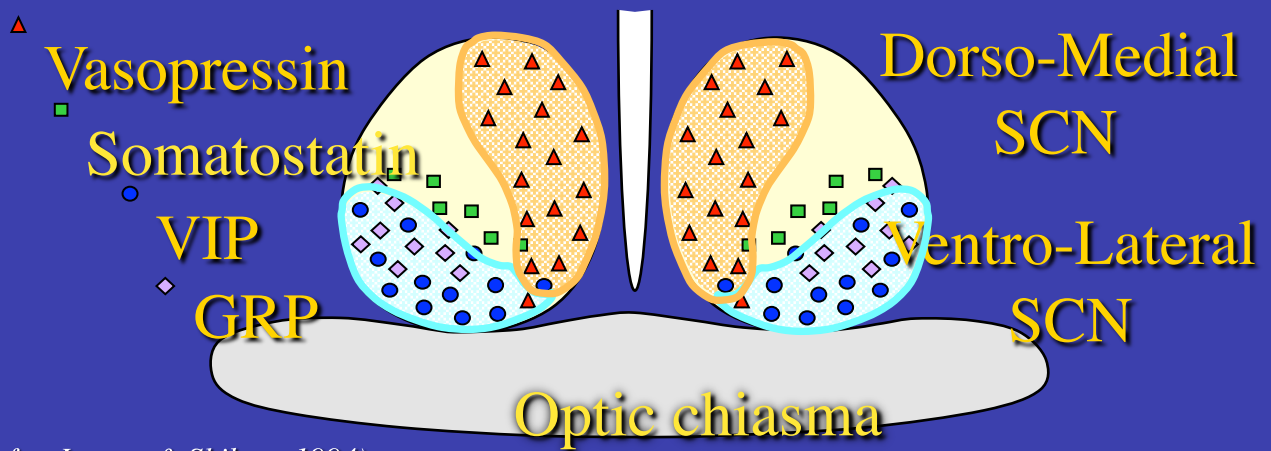


24 h-rhythmic transcription:
10% of genome, among which:
10% : cell cycle
2% : growth factors

(after Hastings, Nature Rev. Neurosci. 2003)

The central circadian pacemaker: the suprachiasmatic (SCN) nuclei



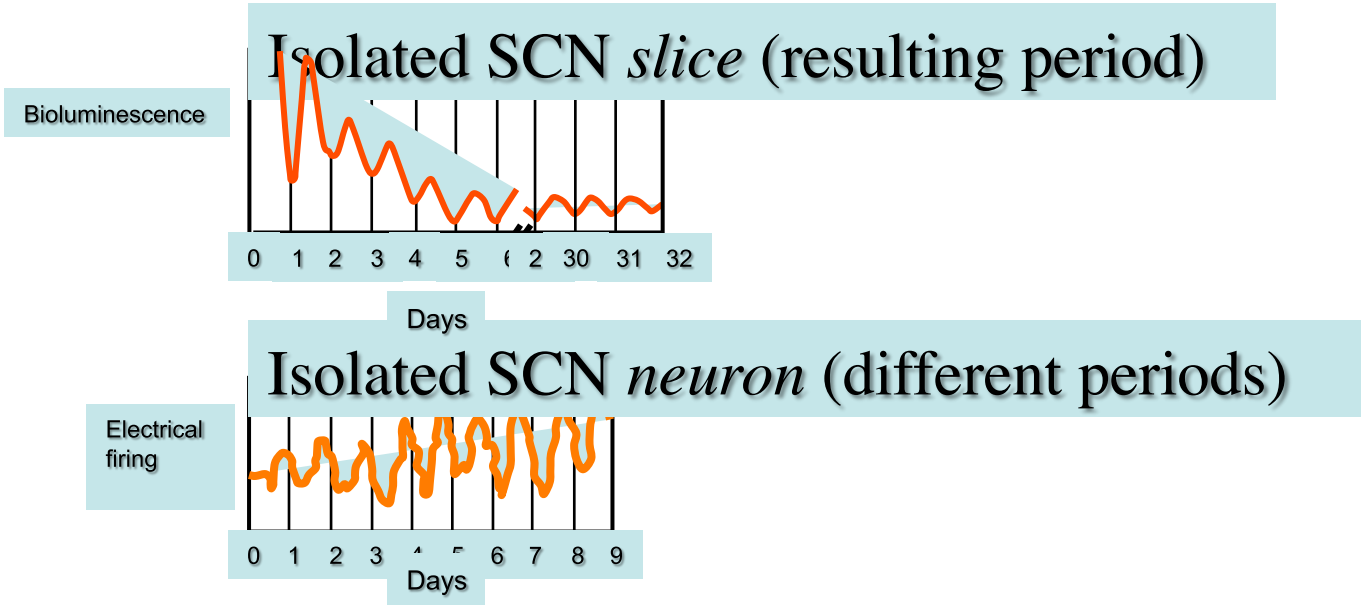


(after Inouye & Shibata 1994)

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)

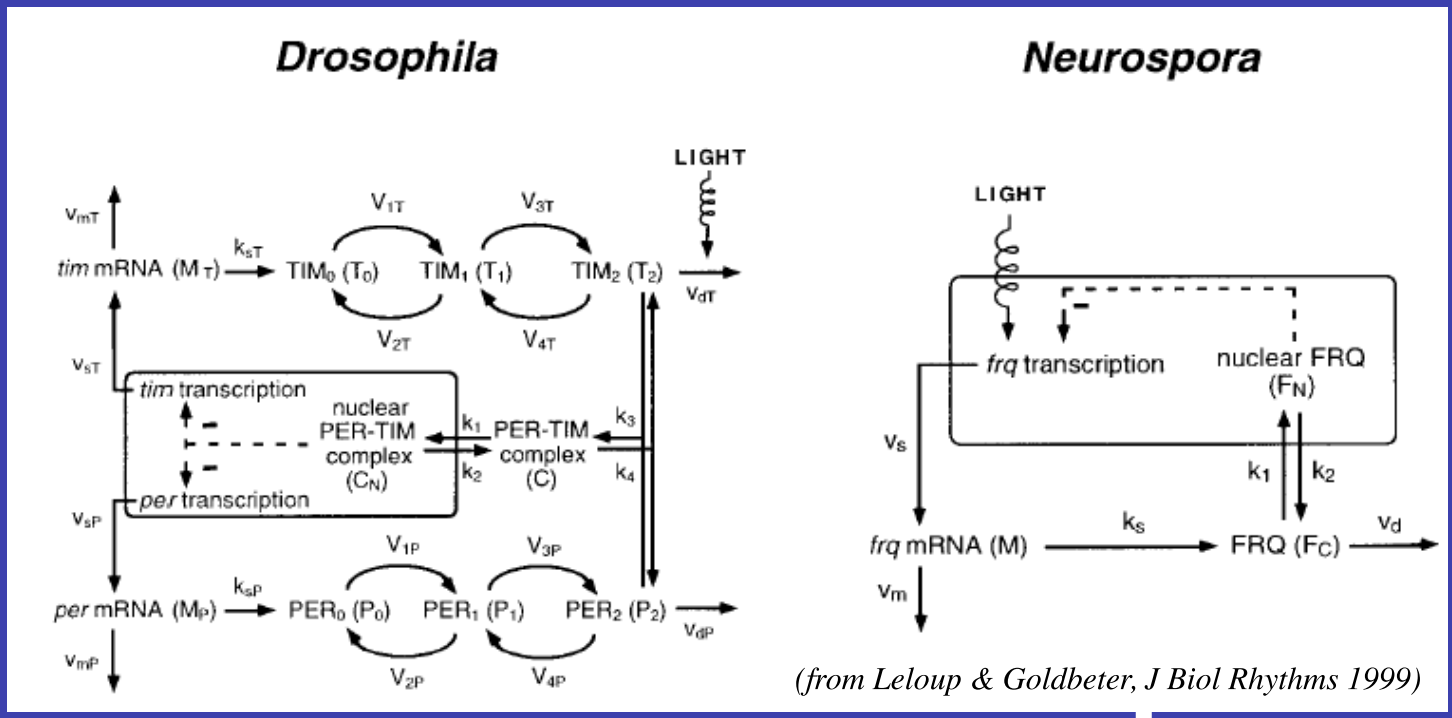
Light entrains the SCN pacemaker but is *not mandatory* for its rhythmic firing

ODE models of the circadian clock

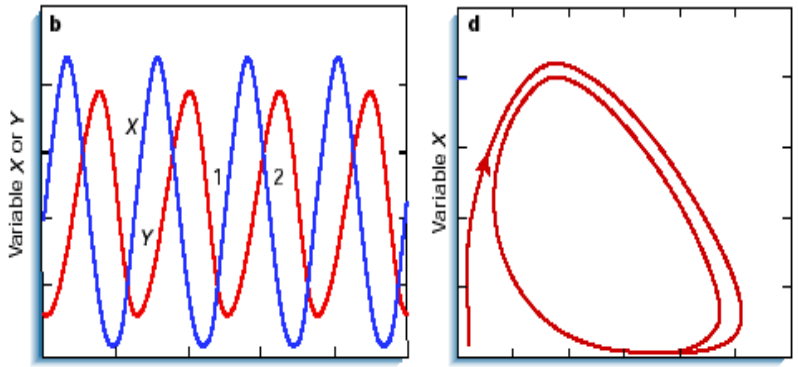
- Goodwin (1965): 3 variables, enzymatic reactions, one sharp nonlinearity
- Forger & Kronauer (2002): Van der Pol-like model, 2 variables
- [Leloup &] Goldbeter (1995, 1999, 2003): 3 (Neurospora *FRQ*); 5 (Drosophila *PER*); 10 (Drosophila *PER+TIM*); 19 (Mammal) variables
- Synchronisation of individual clocks in the SCN: Kunz & Achermann (2003); Gonze, Bernard, Herzel (2005); Bernard, Gonze, Cajavec, Herzel, Kramer (2007)

All these models show (robust) limit cycle oscillations

Simple mathematical models of the circadian clock



(from Leloup & Goldbeter, J Biol Rhythms 1999)



Stable limit cycle oscillations

(Goldbeter, Nature 2002)

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

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

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

Transcription

↓

Translation

↓

Inhibition of transcription

Modelling the SCN as a network of coupled oscillators:

diffusive (electric?) coupling between neurons

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

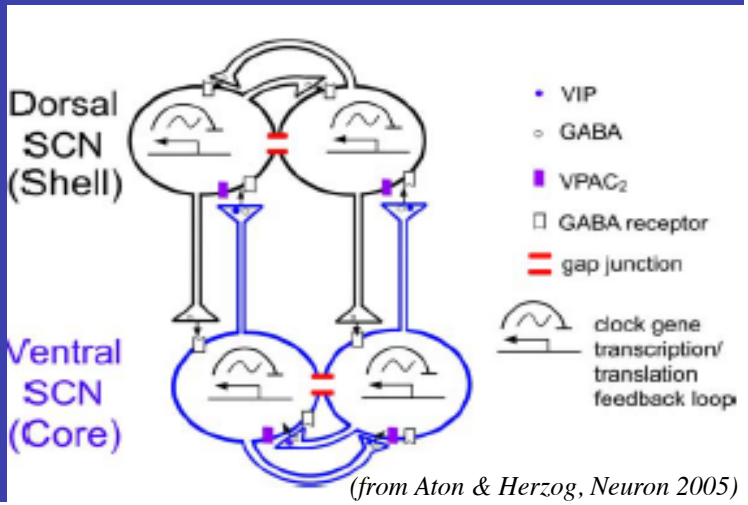
$$\frac{dPER(i)}{dt} = k_s mRNA(i) - V_d \frac{PER(i)}{K_d + PER(i)} - k_1 PER(i) + k_2 Z(i) + K_e \sum_{j \neq i} [PER(j) - PER(i)]$$

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

(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.

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)

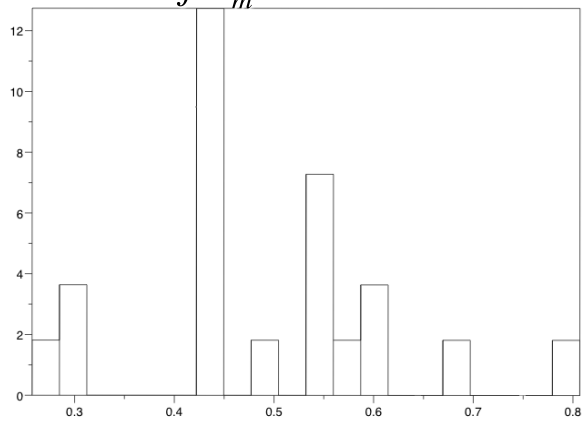
A hue of stochasticity in the model: heterogeneity of endogenous clock periods to be represented by

$V_m = 0.505 + dispersion \,.\, rand('normal')$

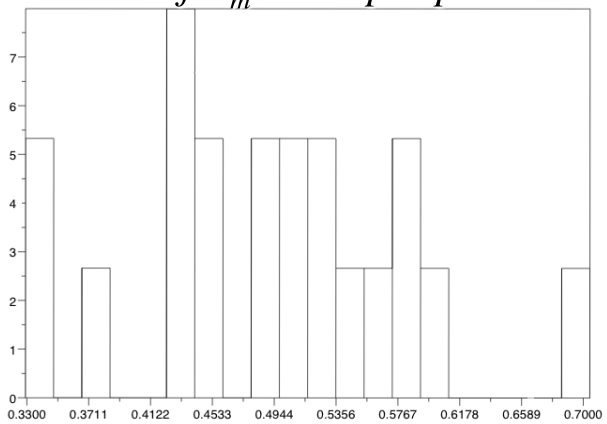
(where $V_m = 0.505 \rightarrow T = 21\ h\ 30$)

Example:

Distribution of V_m in the central clock



Distribution of V_m in the peripheral clock



Plus entrainment by light: L=[0/1] . light, and $V_s = 1.6*(1+L*\cos(2\pi\, t/24))$, hence *entraining period = 24 h;* other: K_e=0.01, light=0.5, dispersion=0.1

Pathways from the SCN toward periphery

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

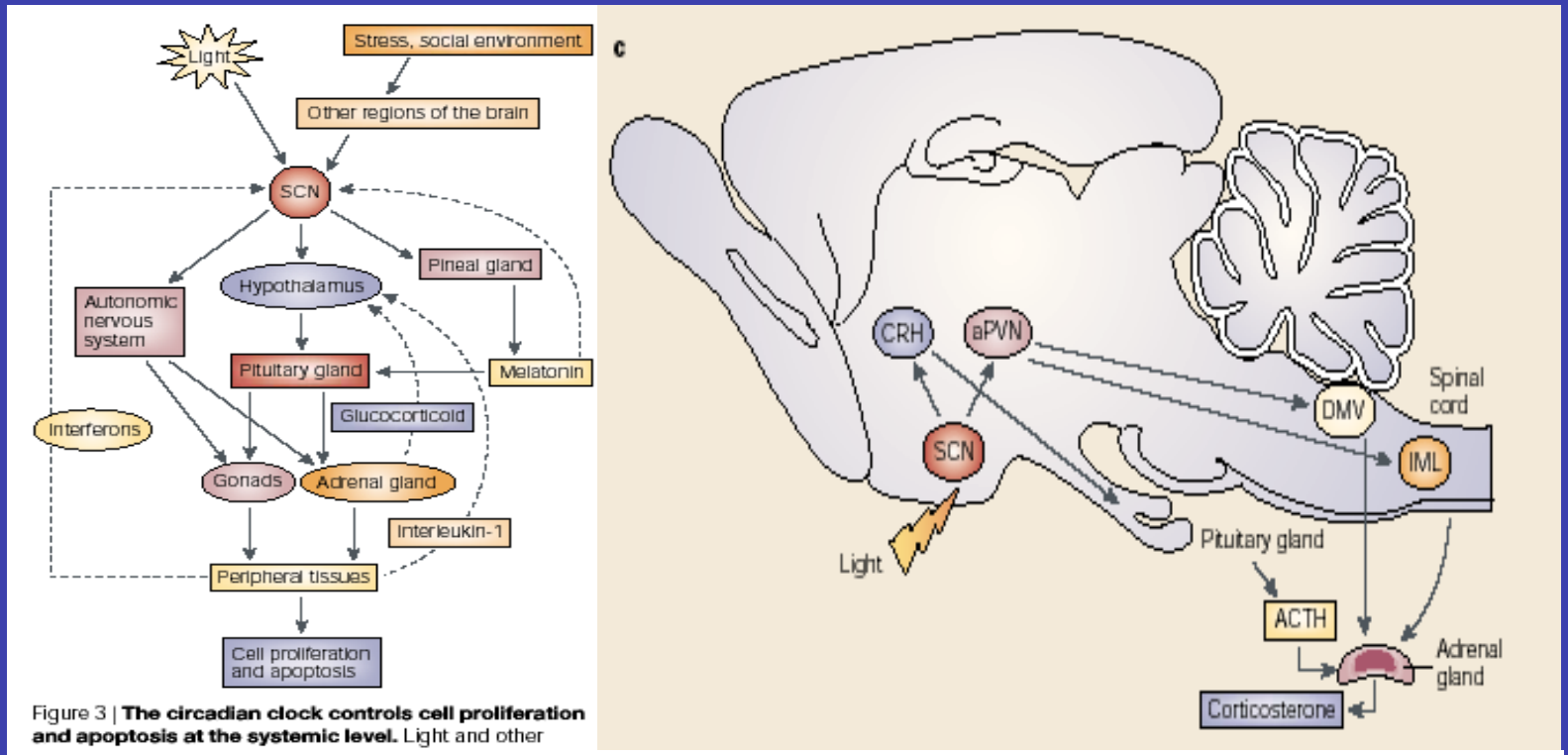


Figure 3 | **The circadian clock controls cell proliferation and apoptosis at the systemic level.** Light and other

(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

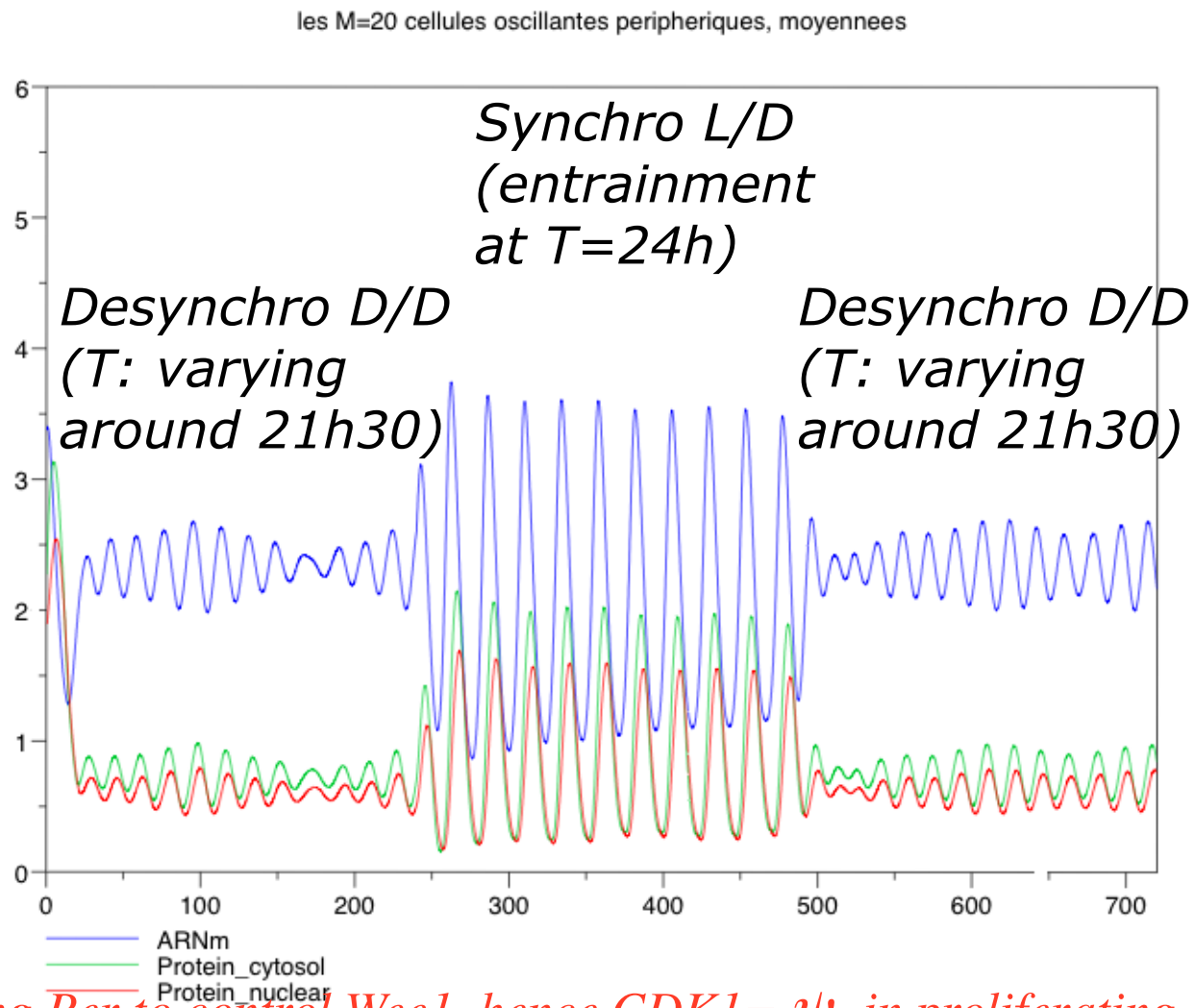
$$\frac{dARN_m}{dt} = V_s \frac{K^n}{K^n + Z^n} - V_m \frac{ARN_m}{K_m + ARN_m}$$

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

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

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

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



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

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

Circadian rhythm and tumour growth: challenging modelling and mathematical questions coming from biological experiments

Circadian rhythm disruption in Man: Loss of synchrony between molecular clocks?

- Circadian desynchronisation (loss of rythms of temperature, cortisol, rest-activity cycle) is a factor of poor prognosis in the response to cancer treatment
  (Mormont & Lévi, Cancer 2003)

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

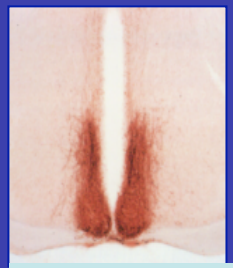
- …effects that are analogous to those of chronic « jet-lag » (photic entrainment phase advance or delay) on circadian rhythms, known to enhance tumour growth *(Hansen, Epidemiology 2001; Schernhammer, JNCI 2001, 2003; Davis, JNCI 2001, Canc Causes Control 2006)*

- *…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?*

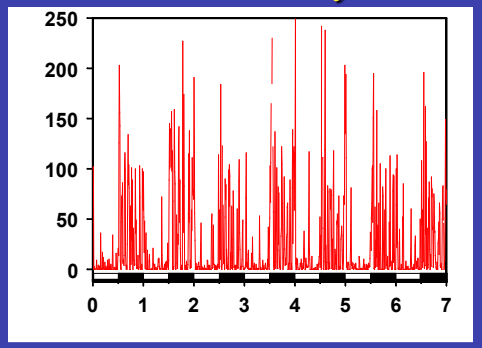
- […and hence resynchronisation therapies (by melatonin, cortisol) in oncology??]

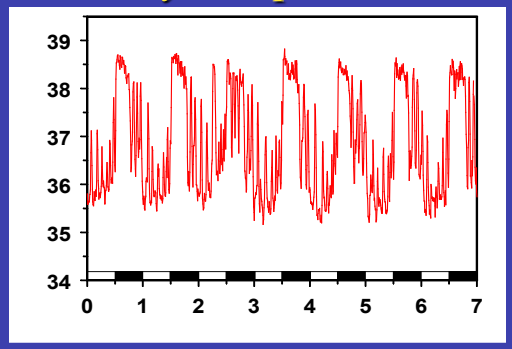
Circadian rhythm disruption in mice

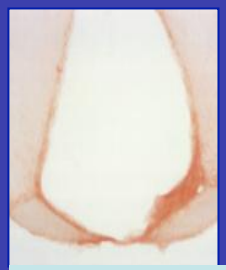
Rest-activity *Body temperature*



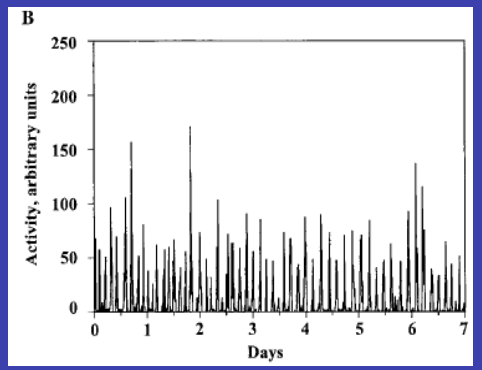
Intact SCN

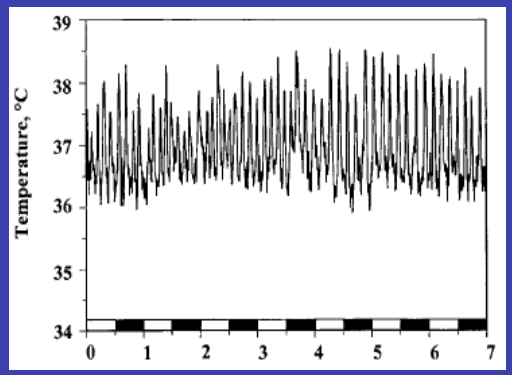


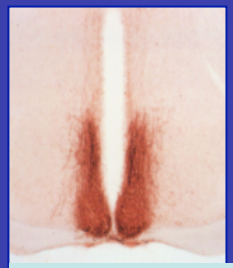




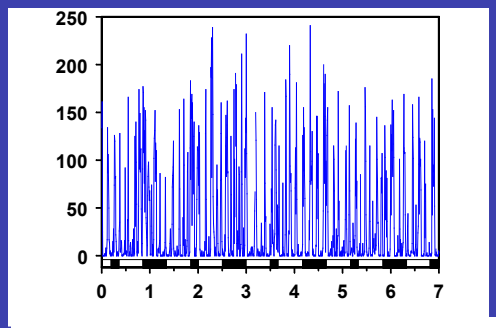
Electrocoagulation

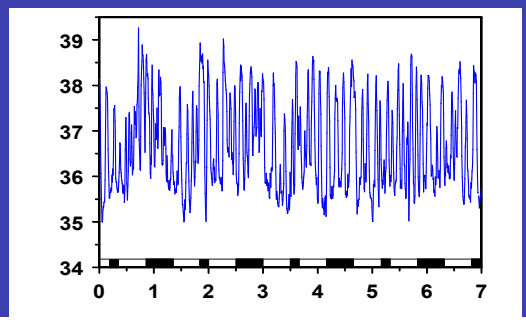






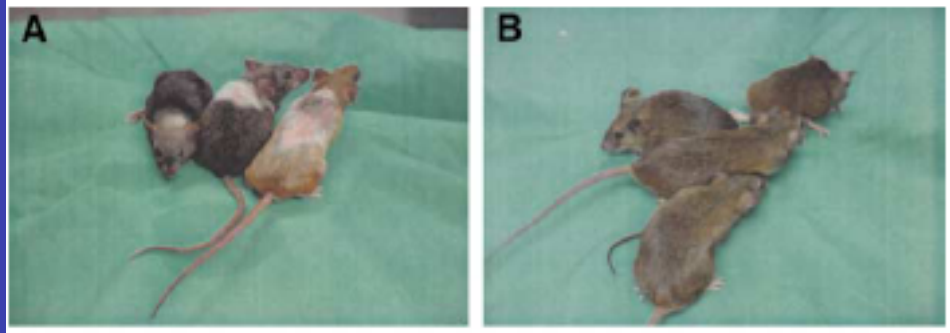
Intact+Jet-lag





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

Circadian rhythm and cancer growth in mice



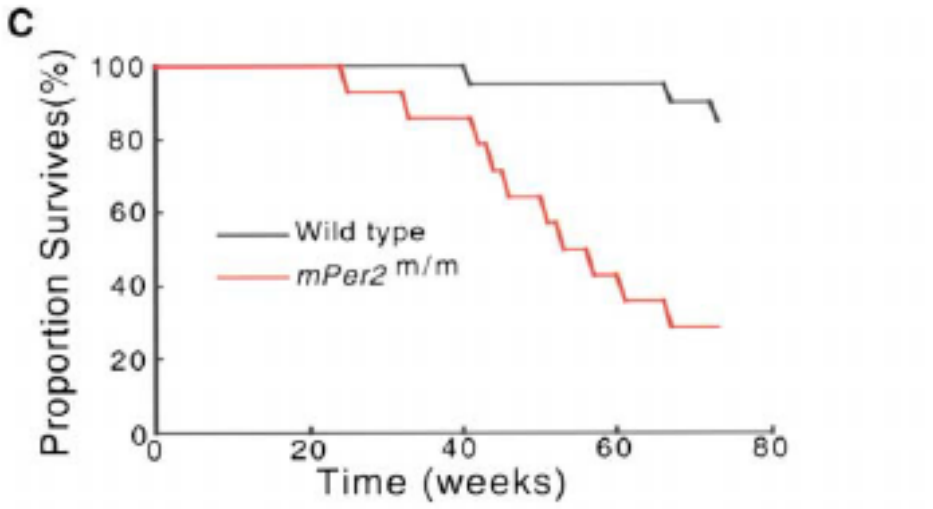
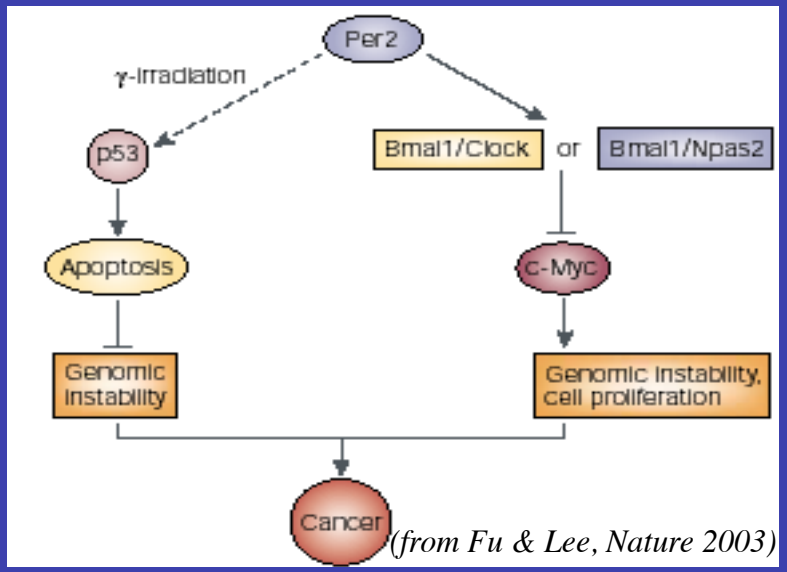


Figure 2. *mPer2*$^{m/m}$ Mice Show Increased Sensitivity to γ Radiation
(A) All the irradiated *mPer2*$^{m/m}$ mice show hair graying at 22 weeks after irradiation. Some of them also show hair loss on the back.
(B) Wild-type mice at 22 weeks after irradiation.
(C) Survival curve for wild-type and *mPer2*$^{m/m}$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

(from Fu et al., Cell 2002)

NB: Per2 is a gene of the circadian clock

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



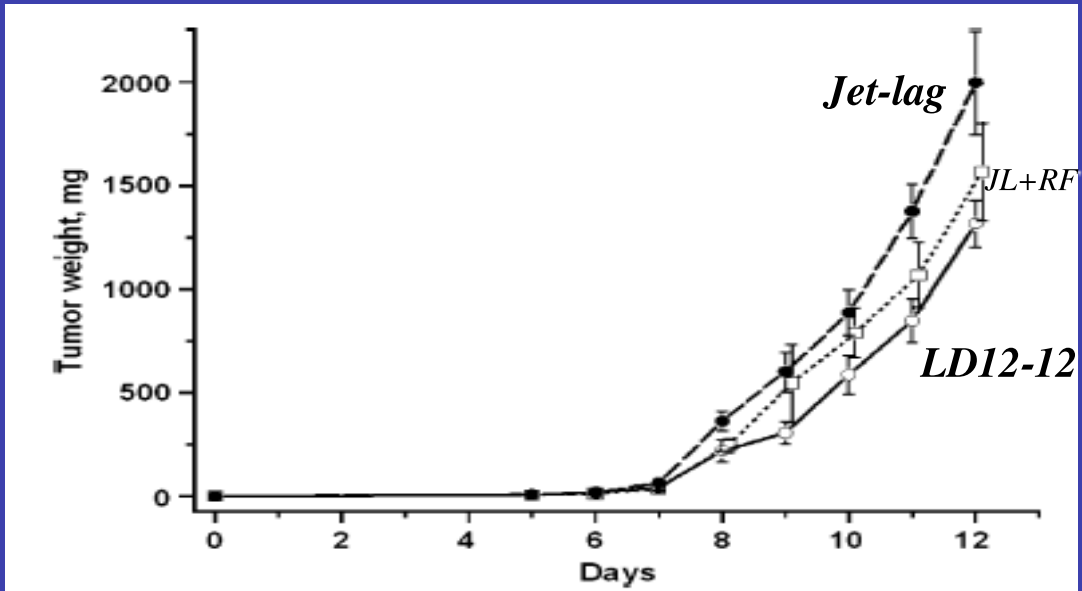
(from Fu & Lee, Nature 2003)

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

Observation: a circadian rhythm perturbation by chronic jet-lag-like light entrainment (8-hour phase advance every other night) enhances GOS tumour proliferation in mice

LD12-12

Jet-lag



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

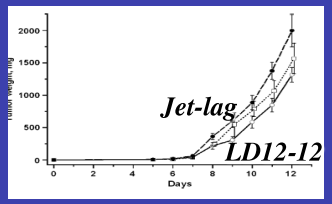
Here, clearly:
λ(Jet-lag) > λ(LD 12-12)
if λ is a growth exponent

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)

Mathematical formulation of the problem, first approach

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



λ(Jet-lag) > λ(LD 12-12)

Instead of the initial eigenvalue problem with time-periodic coefficients:

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

Define the stationary system with constant [w. r. to time t] 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 d_i only

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

Theorem (B. Perthame, 2006):

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

[Proof by a convexity argument (Jensen's inequality)]

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

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

…But no such clear hierarchy exists if the control is exerted on the sole transition functions $K_{i->i+1}$

(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 phase 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

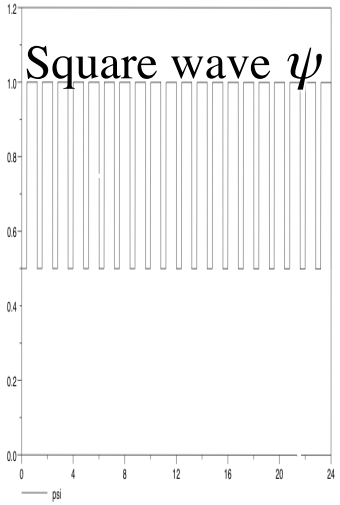
ψ_2: G2/M gate open 12 h / closed 12 h

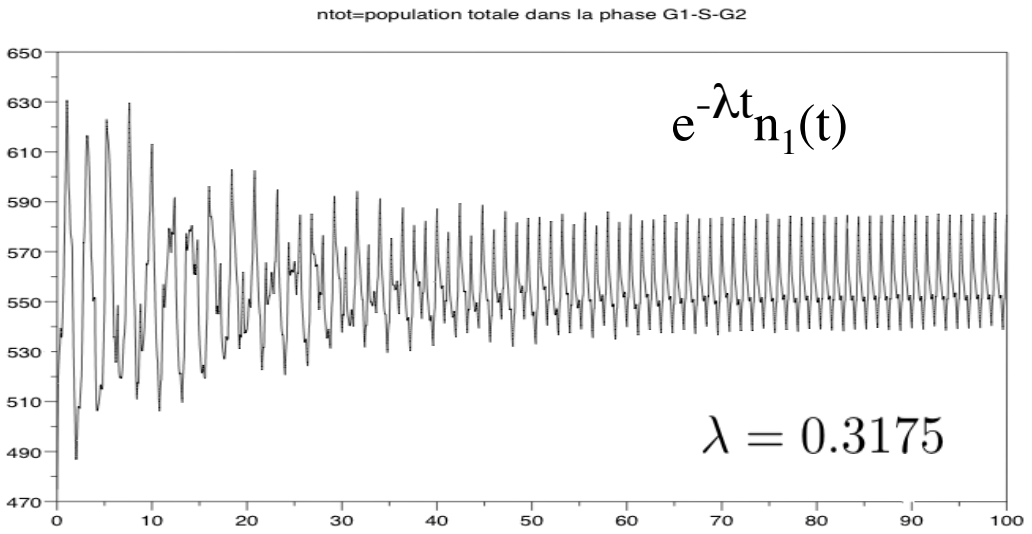
(G1-S-G2/M)	(periodic)	(constant)
time ratio, ψ_2	λ_{per}	λ_{stat}
1	0.2623	<u>0.2821</u>
2	0.3265	<u>0.3448</u>
3	...	...
4	...	...
5	...	...
6	...	...
7	0.4500	<u>0.4529</u>
8	<u>0.4588</u>	0.4575
10	<u>0.4713</u>	0.4641
20	<u>0.5006</u>	0.4818

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

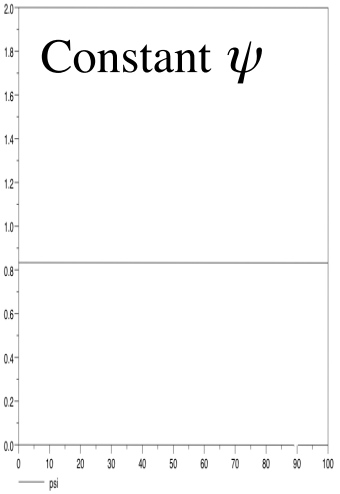
Example: ψ=1(16h)+.5(8h) sq. wave vs. constant (=no) control

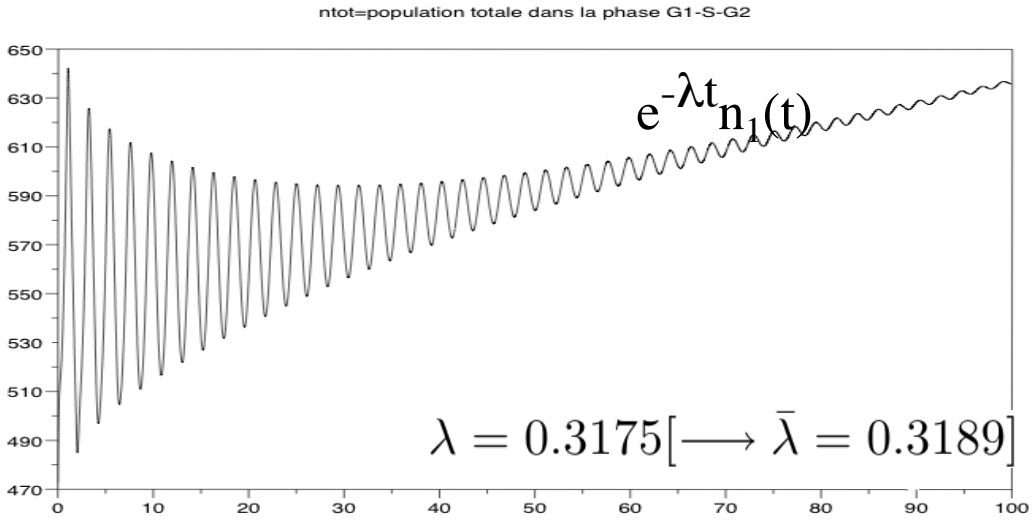
Two phases





Two phases





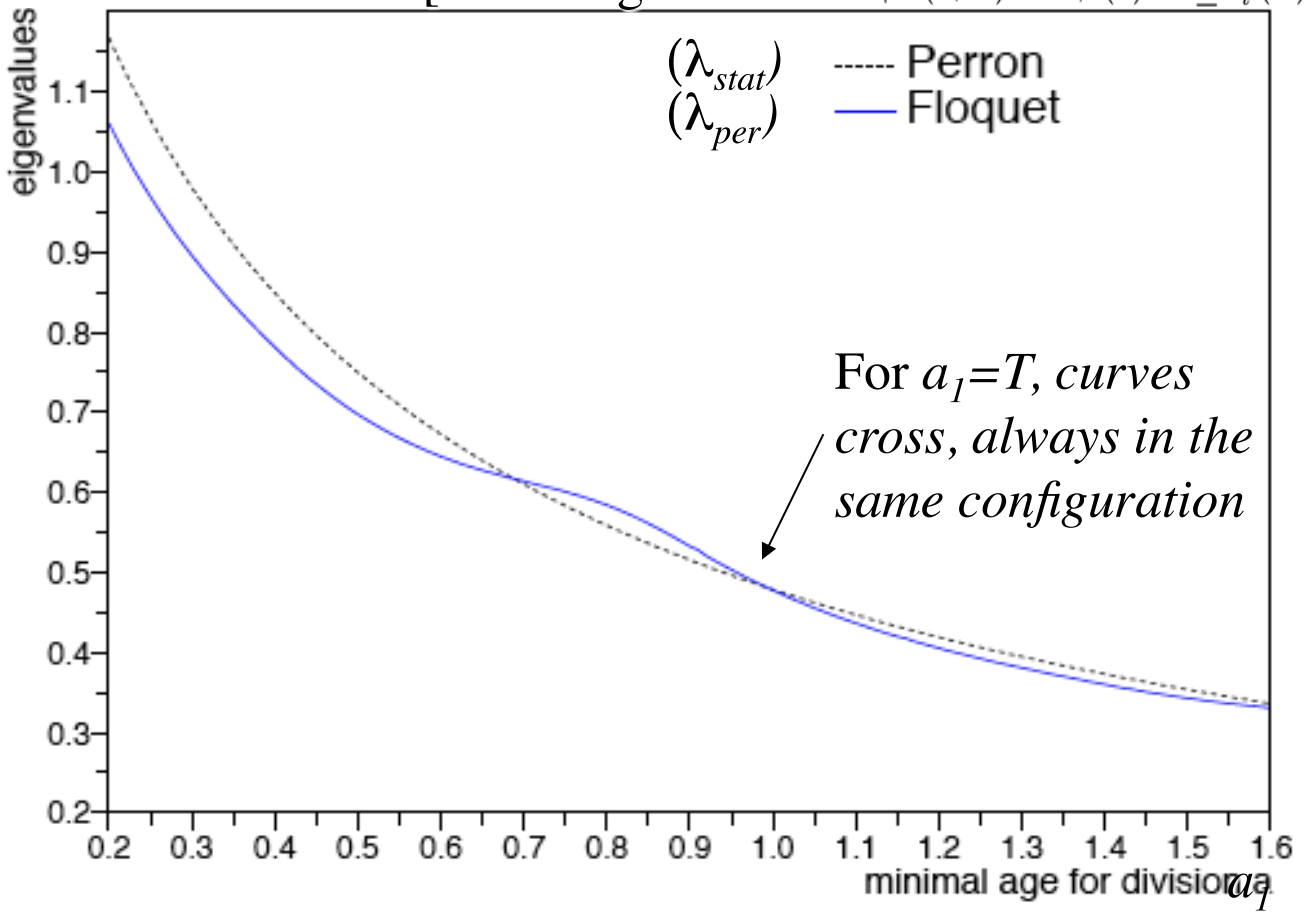
(Here: 2 cell cycle phases of equal duration, control exerted on G_2/M transition)

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

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

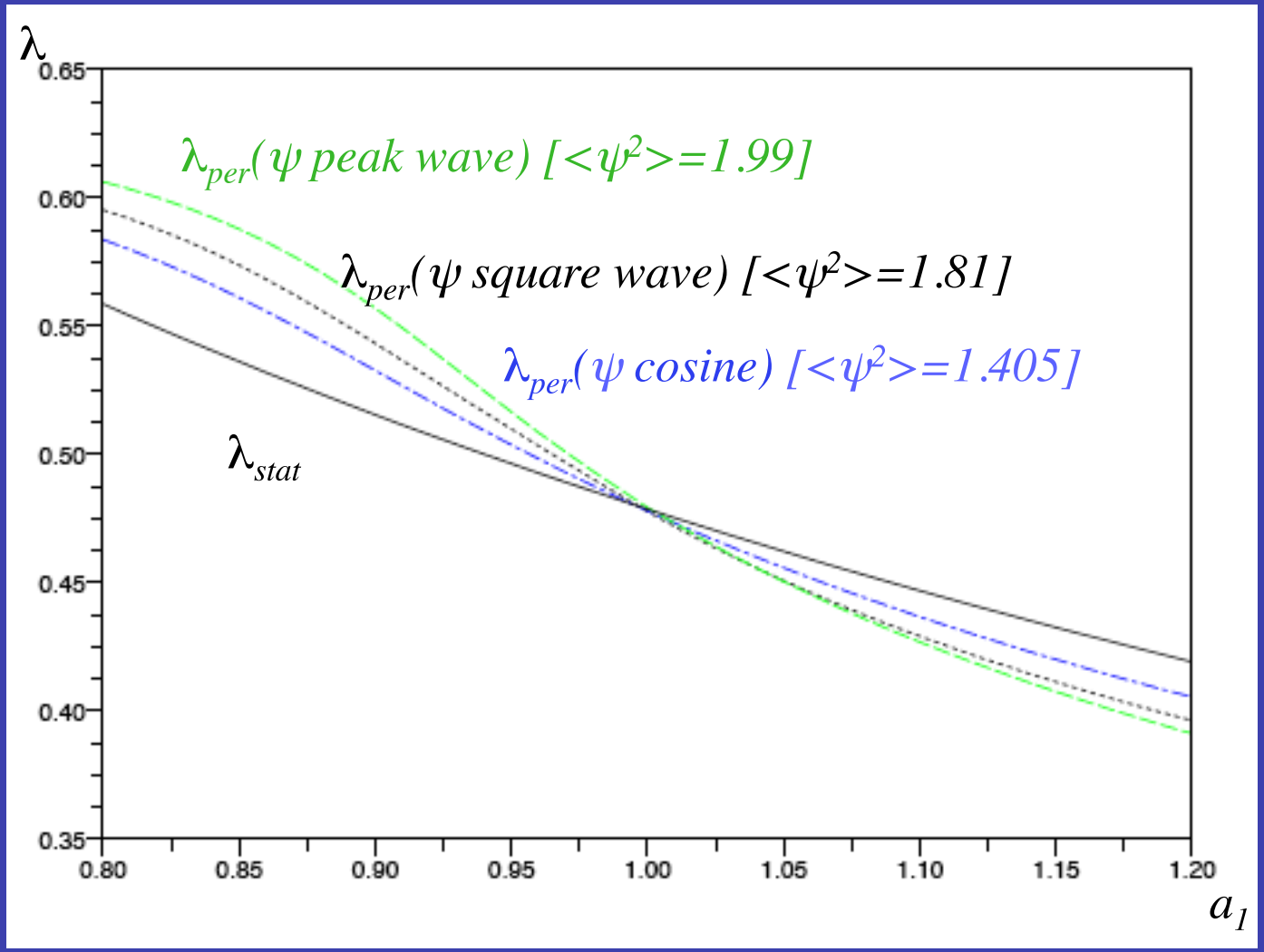
proof for a 1-phase model [illustrated here with control $\psi(\tau)=1+0.9\cos 2\pi t/T$]

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



(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 (G2/M transition)



(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 $K_{i->i}$

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

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

JC, S. Gaubert, B. Perthame C. R. Acad. Sci. Ser. I (Math.) Paris, 2007; JC, S. Gaubert, Th. Lepoutre MMNP 2009

…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!)

But (new result that generalises the previous one):

Theorem (S. Gaubert , Th. Lepoutre):
Using an even more general model of renewal with periodic control of birth and death rates,

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

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., *(JC, S. Gaubert, T. Lepoutre, MCM 2010)*

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	$\lambda^\theta_F \le \theta\lambda^1_F + (1-\theta)\lambda^2_F$

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

En passant: an application of this convexity result to theoretically justify cancer chronotherapeutics *(Th. Lepoutre)* by *less toxicity on healthy cells* in the periodic control case:

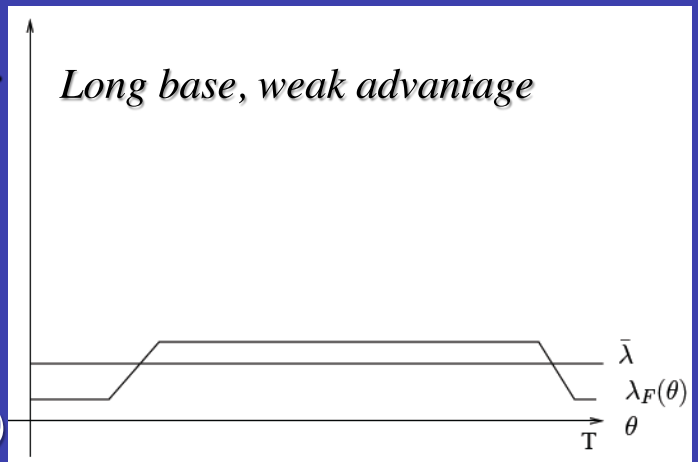
Periodic drug delivery with time shift θ *and action on death rates:* replacing $d_i(t)$ by $d_i(t-\theta)$ *will yield* $\lambda_F(\theta)$ *and if* $\bar{\lambda}$ *is the first eigenvalue corresponding to an averaged death rate, then:*

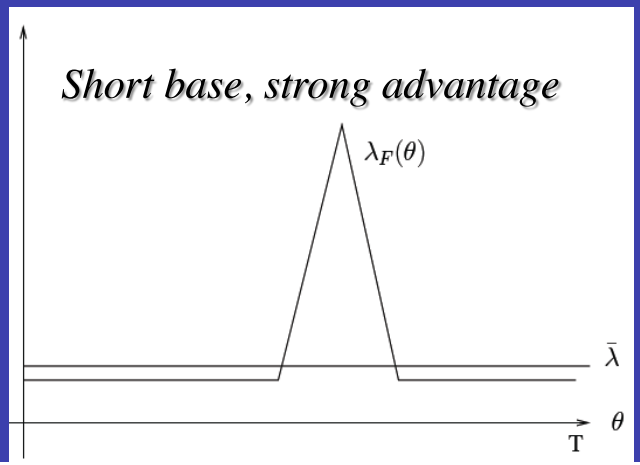
$$\bar{\lambda} \leq \frac{1}{T}\int_0^T \lambda_F(\theta)d\theta$$

i.e., the toxicity of the averaged system (constant delivery) will be higher than the average toxicity of all periodic shifted schedules (θ *=1,…24 h)*

2 graphic examples:

(JC, S. Gaubert, T. Lepoutre, MCM 2010)





Still searching for an explanation,
following alternate tracks:
Just what is disrupted circadian control?

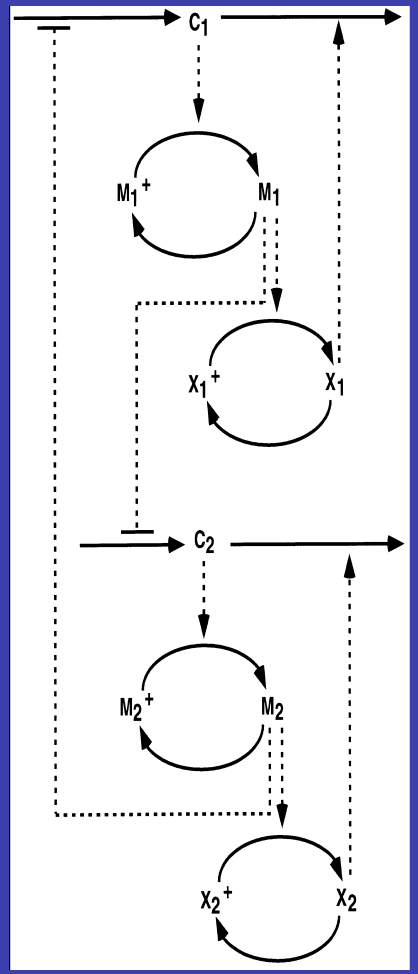
Including more phase transitions in the cell cycle model?

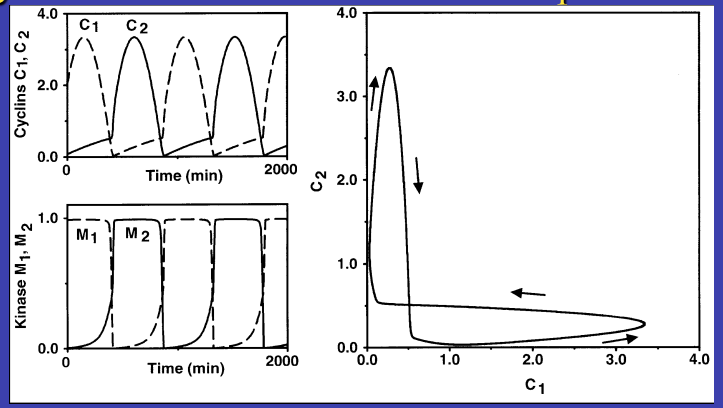
Hint: an existing model for G_1/S and G_2/M synchronisation

(recalling the minimum mitotic oscillator *(C, M, X)* by A. Goldbeter, 1996, here duplicated to take into account synchronisation between G_1/S and G_2/M transitions)

i=1: G_1/S

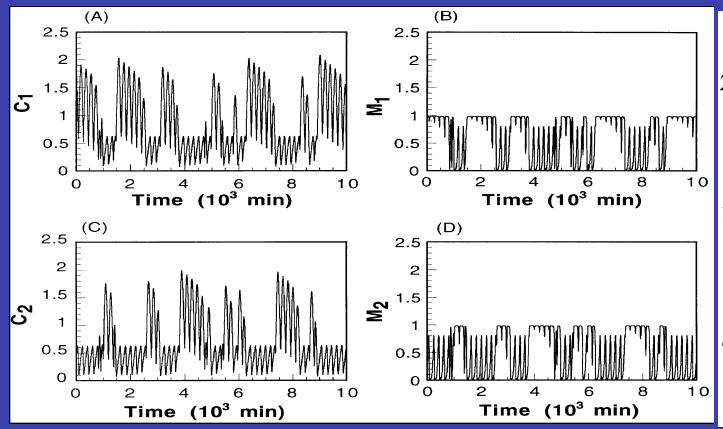
i=2: G_2/M

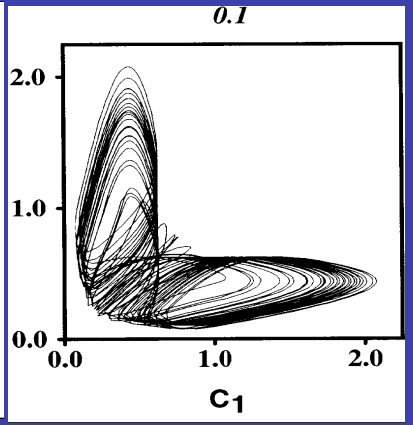




C_i=Cyclin
M_i=CDK
X_i=Protease

Changing the coupling strength may lead to:



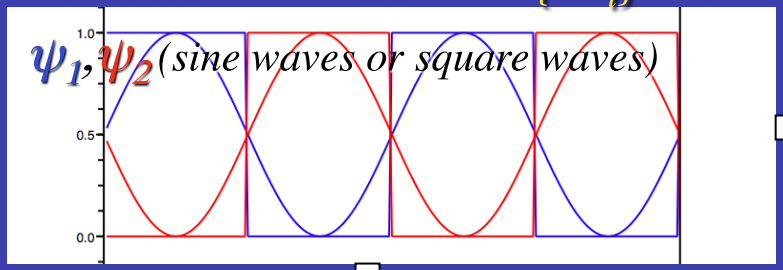


Romond, Gonze, Rustici, Goldbeter, Ann NYAS, 1999

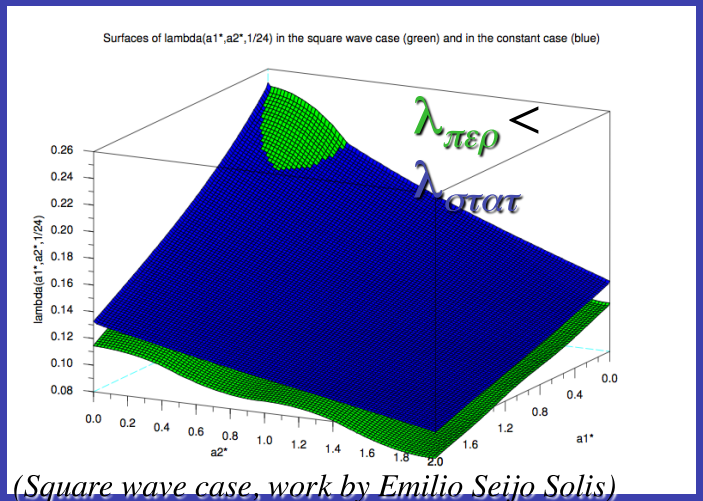
Hence a second approach: Numerical results with phase-opposed periodic control functions ψ_i on transitions G_1/S and G_2/M

Numerical simulations on a *3-phase model* 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., $\psi_3 = 1$).

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



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



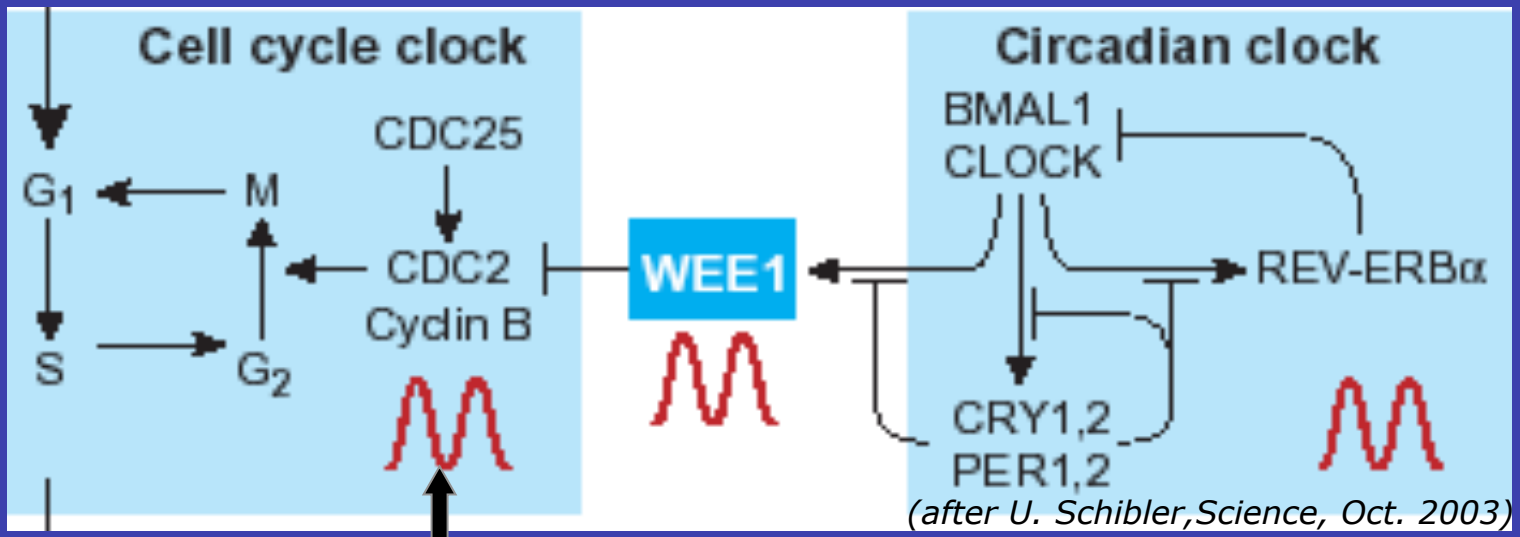
(Square wave case, work by Emilio Seijo Solis)

…more consistent with observations, assuming

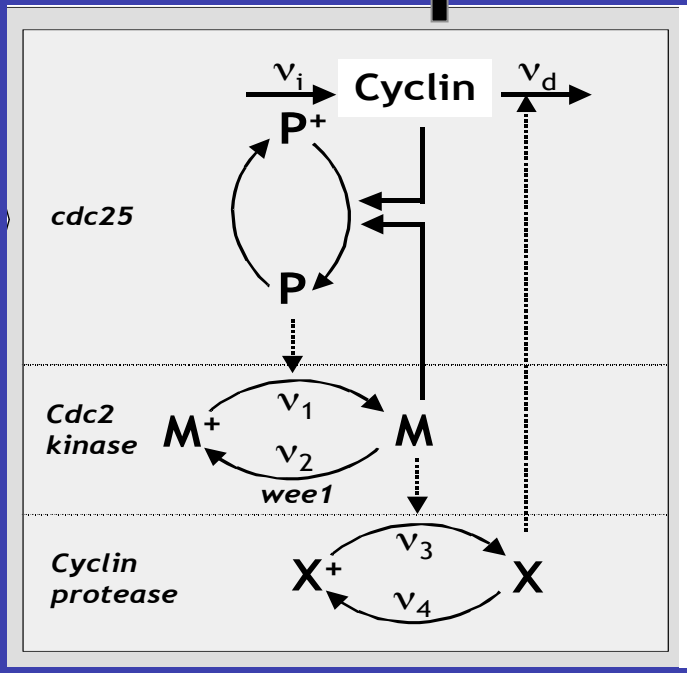
$\lambda(LD\ 12\text{-}12) = \lambda_{\pi\varepsilon\rho} < \lambda_{\sigma\tau\alpha\tau} = \lambda(jet\text{-}lag)$

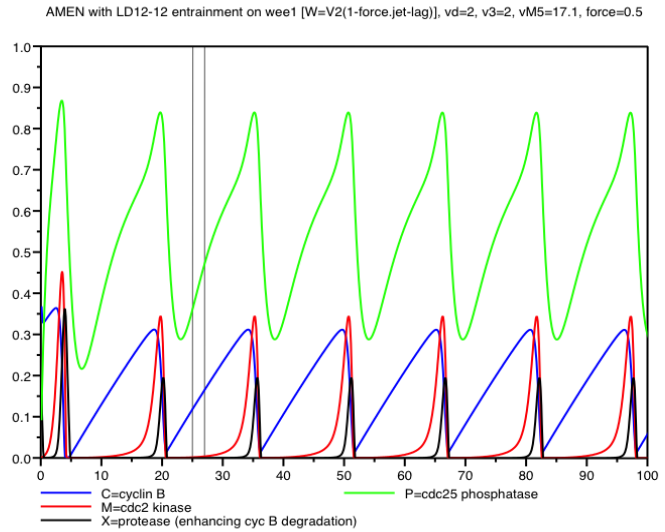
(jet-lag=desynchronisation between clocks?)

Another track: a molecular connection between cell cycle and clock: Cdk1 opens G2/M gate; Wee1 inhibits Cdk1



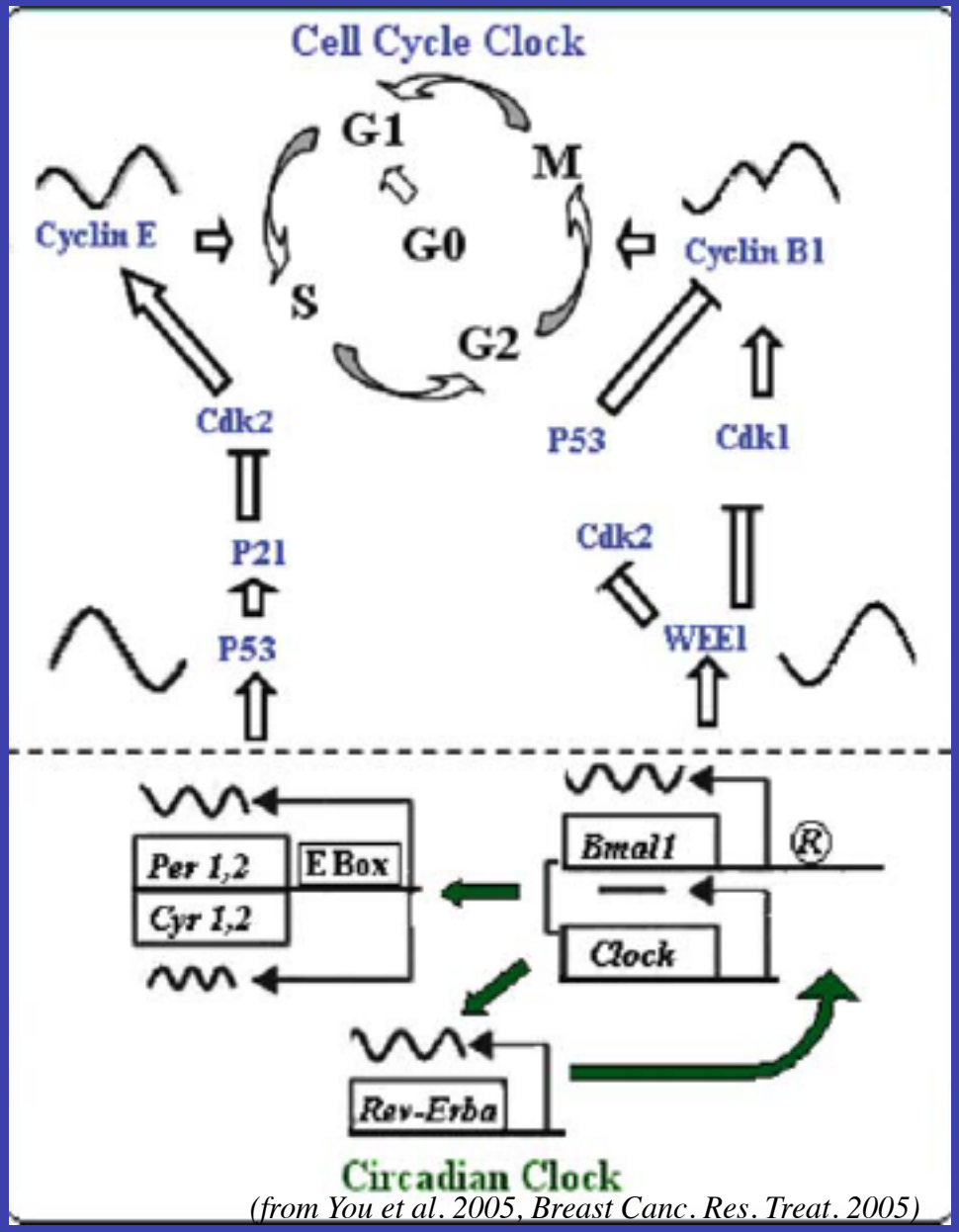
(after U. Schibler,Science, Oct. 2003)





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

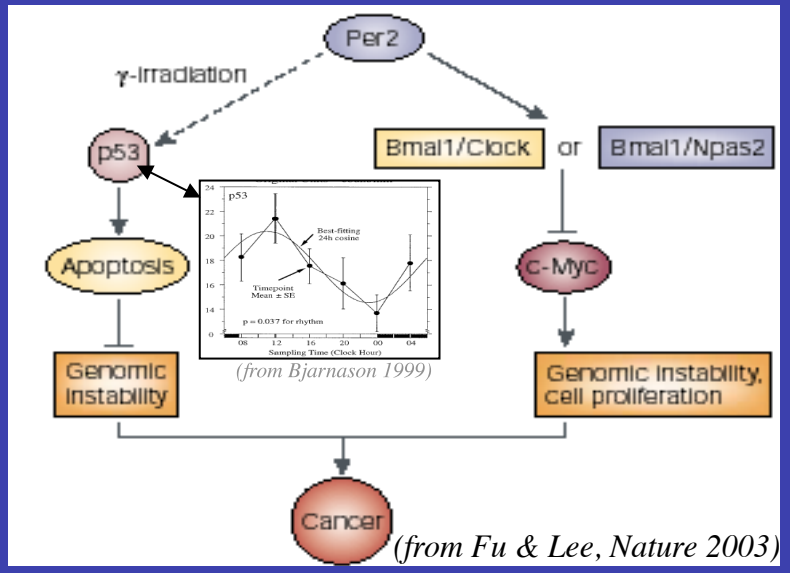
More connections between the cell cycle and circadian clocks



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

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

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

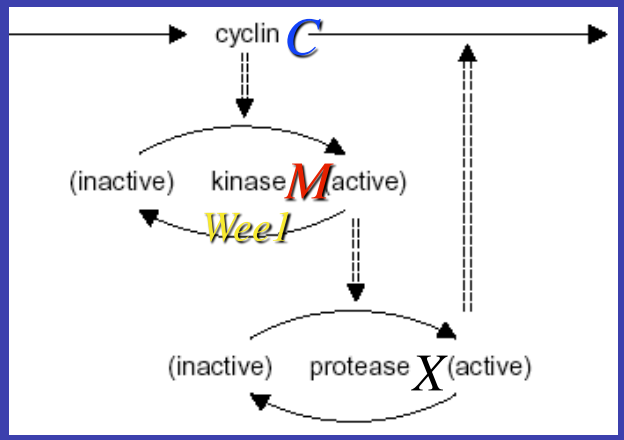


(from Bjarnason 1999)

(from Fu & Lee, Nature 2003)

Relating circadian clocks with the cell cycle: G_2/M

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



Wee1

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

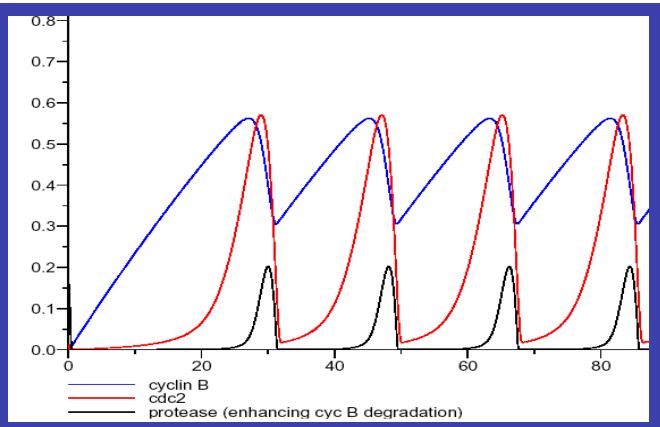
$$\frac{dM}{dt} = v_1 \frac{C}{K_c + C} \frac{(1 - M)}{K_1 + (1 - M)} - V_2 \frac{M}{K_2 + M},$$

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

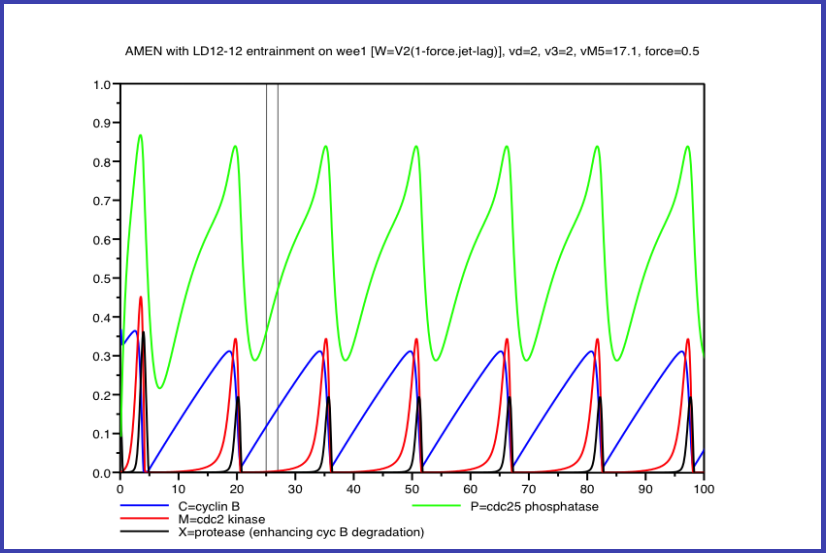
C = cyclin B, M = cyclin dependent kinase cdk1, X = degrading protease

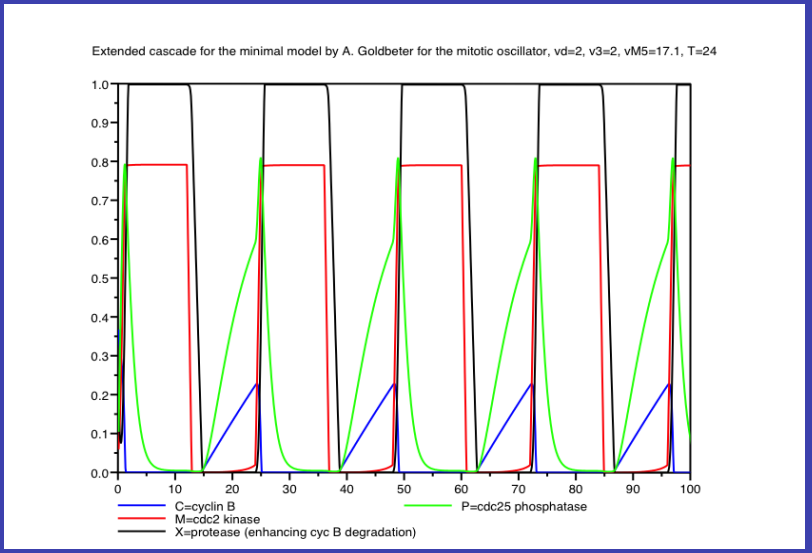
Input: *Per=Wee1*; output: M=Cdk1=ψ
Switch-like dynamics of dimer Cyclin B-cdk1
Adapted to describe G_2/M phase transition

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



Control on transition rate G2/M: Cdk1, entrained by Wee1





A. Goldbeter's model (1997), cdc[=Cdk1] entrained by 24 h-rhythmic Wee1

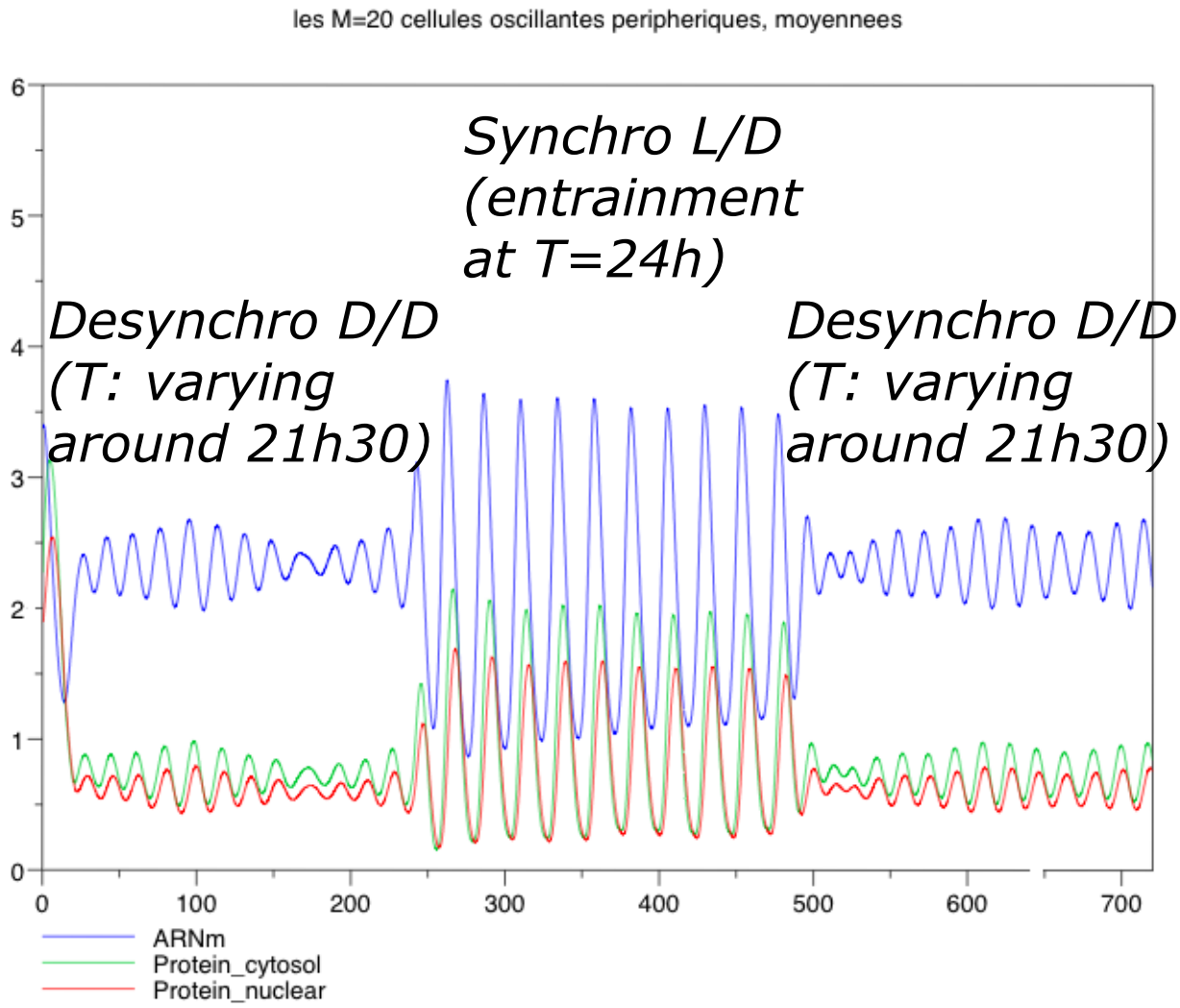
Same model, Wee1=constant, coefficients set to yield 24 h period

Template: square wave
4 h x 1 and 20 h x zero

Template: square wave
«LD 12-12-like»: 12 h x 1, 12 h x zero

…or constant control

Hence a third (molecular) approach: a disrupted clock? *peripheral* averaged clock 1) without *central* entrainment by light; 2) with; 3) without

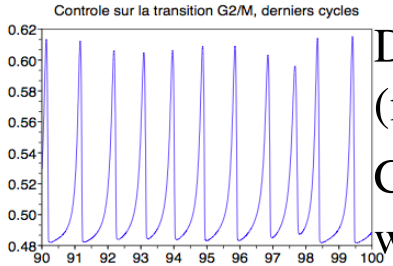


Resulting Per to control Wee1, hence CDK1= ψ, in proliferating cells

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

Clock perturbation and cell population growth

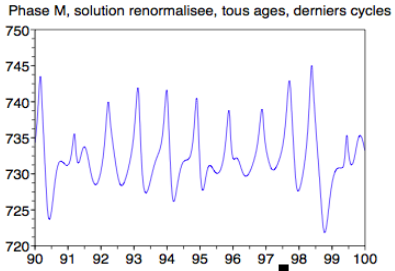
Wee1 oscillators synchronised or not in a circadian clock network model



Desynchronised Wee1 (no entrainment by light):

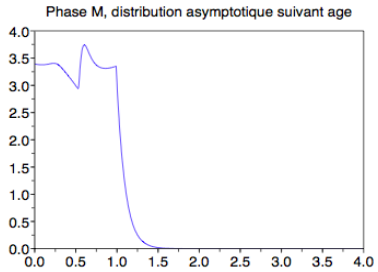
Control Cdk1= ψ with perturbed clock

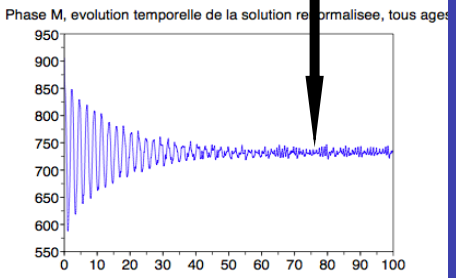
Resulting irregular cell population dynamics in M phase →

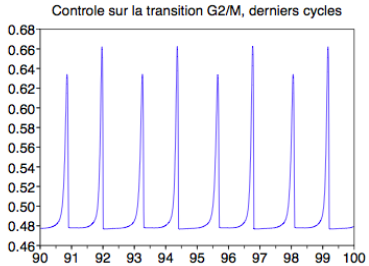


Wee1 is desynchronised at the central (NSC) level

Resulting λ=0.0466



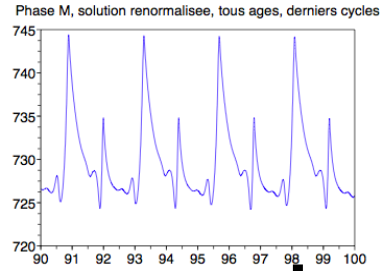




Synchronised Wee1 (entrainment by light):

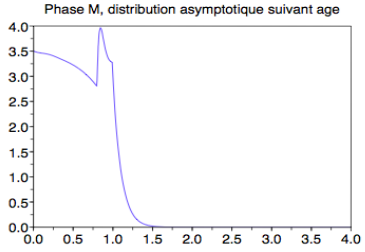
Control Cdk1= ψ with unperturbed clock

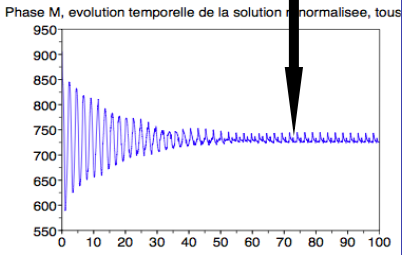
Resulting regular cell population dynamics in M phase →



Wee1 is synchronised at the central (NSC) level

Resulting λ=0.0452

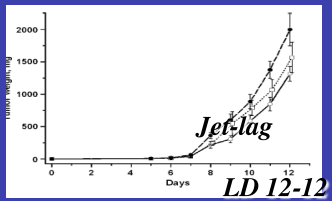




Still a general mathematical formalism to describe and analyse circadian disruption is wanted…

Fourth approach: What if we had it all wrong from the very beginning?

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 (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, insensitive (or less sensitive) to circadian messages, just proliferate unabashed: …*a story to be continued!*

[Temporary] Conclusion

- Searching for an explanation to the initial biological observation, we have come across different (and contradictory) reasons why it should be so.

- Biological evidence is still lacking to make us conclude in favour of one explanation or another (disrupted clock: a proliferative advantage or drawback? …For which cell populations?).

- A 'by-product' of our quest is a new convexity result on the periodic control of a general renewal equation, that can also be interpreted in favour of the concept of chronotherapy as compared with classical constant infusion therapies in oncology.

Molecular pharmacokinetics-pharmacodynamics (PK-PD)

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

1st example: Modelling molecular PK-PD of *Oxaliplatin*: a model involving DNA damage, GSH shielding and repair

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

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

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

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

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

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

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

Molecular PK of oxaliplatin: plasma compartment

Mass of active oxaliplatin

Constant clearance

Instantaneous infused dose (flow)

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

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

ρ_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

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

Plasma protein synthesis shows circadian rhythm

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 and reduced glutathione GSH [=G] to bind oxaliplatin in proliferating cells

Molecular PD of oxaliplatin activity in tissue

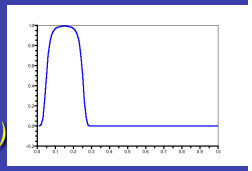
Mass of free DNA

Action of oxaliplatin on free DNA (F)

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

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

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

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

Activity of γ-Glu-cysteinyl ligase (GCS)

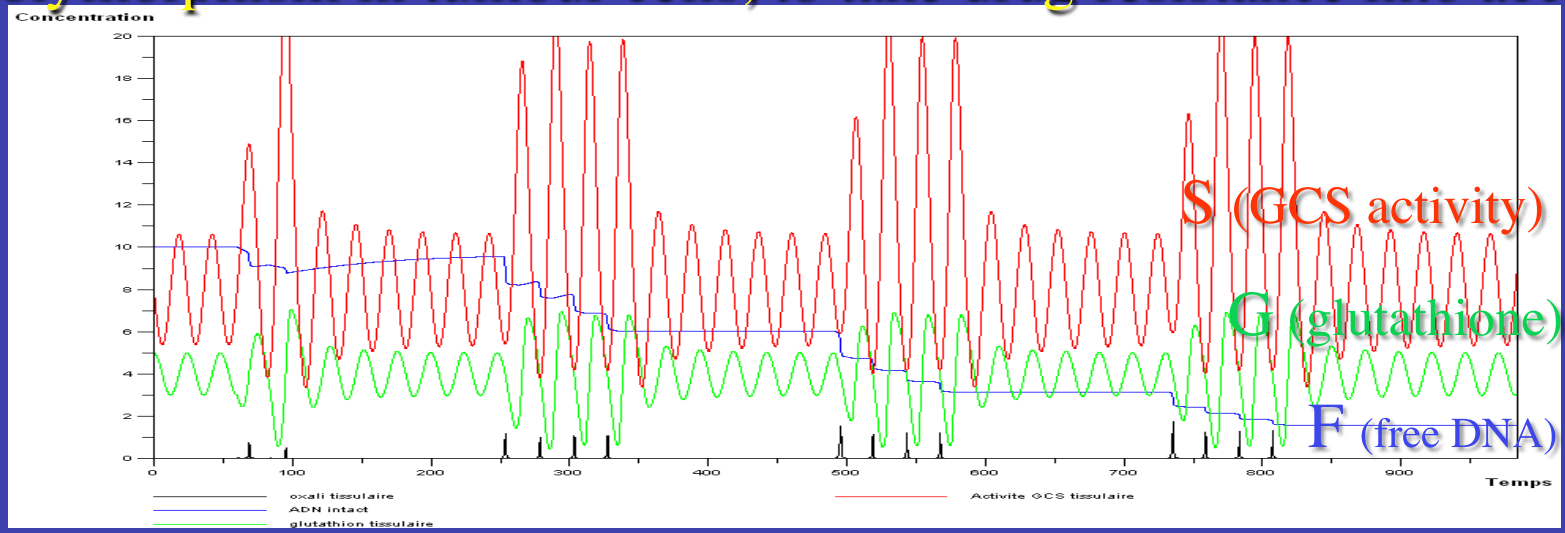
ω_G tunes the period of the cycle of GSH synthesis by GCS

Glutathione synthesis (=detoxification) in cells shows circadian rhythm

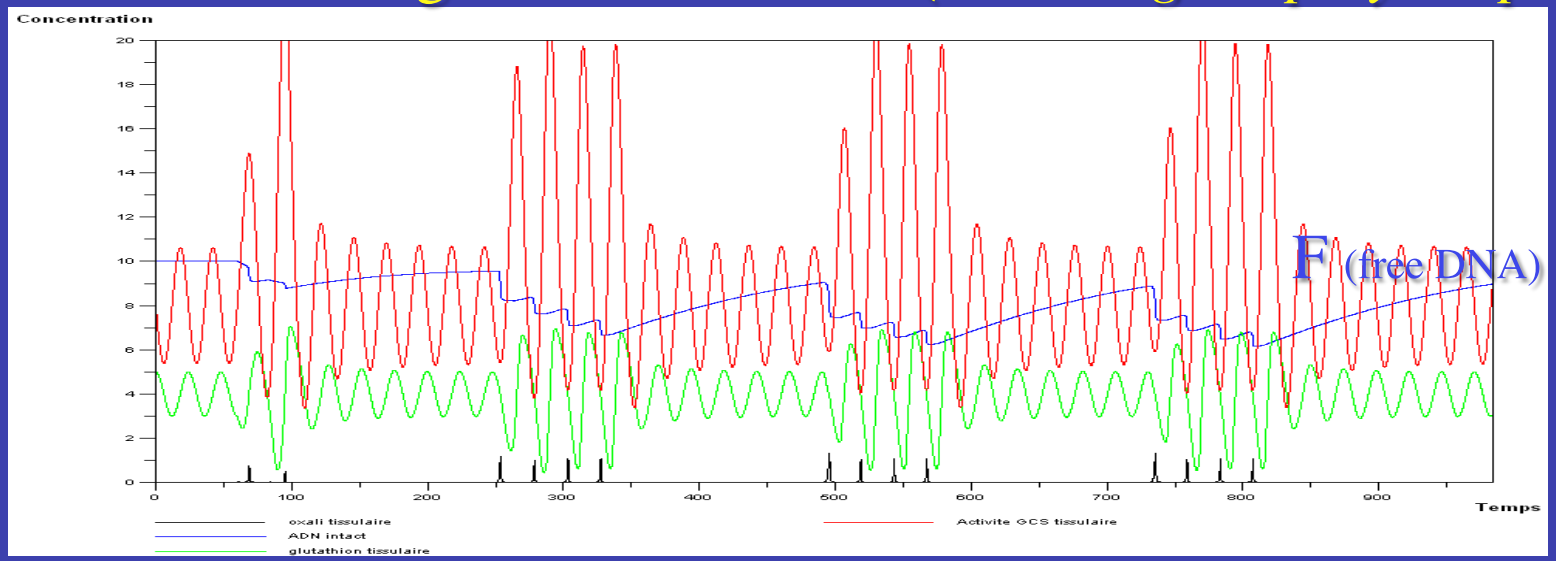
$$\frac{dS}{dt} = -\frac{\omega_G^2}{\delta}(G - G_0)$$

$1\text{-}F/F_0 \longrightarrow$ *cell death*

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

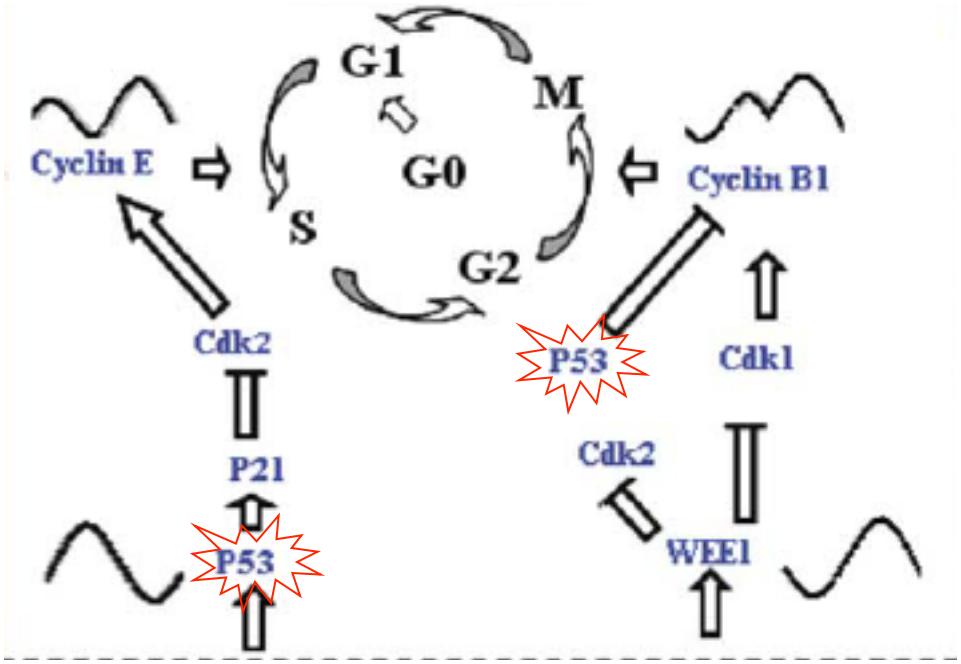


…the same with stronger MMR function (ERCC2 *gene polymorphism*):



(Diminished V_{GST} binding to GSH / cellular uptake ξ, instead of enhanced repair, lead to comparable results)

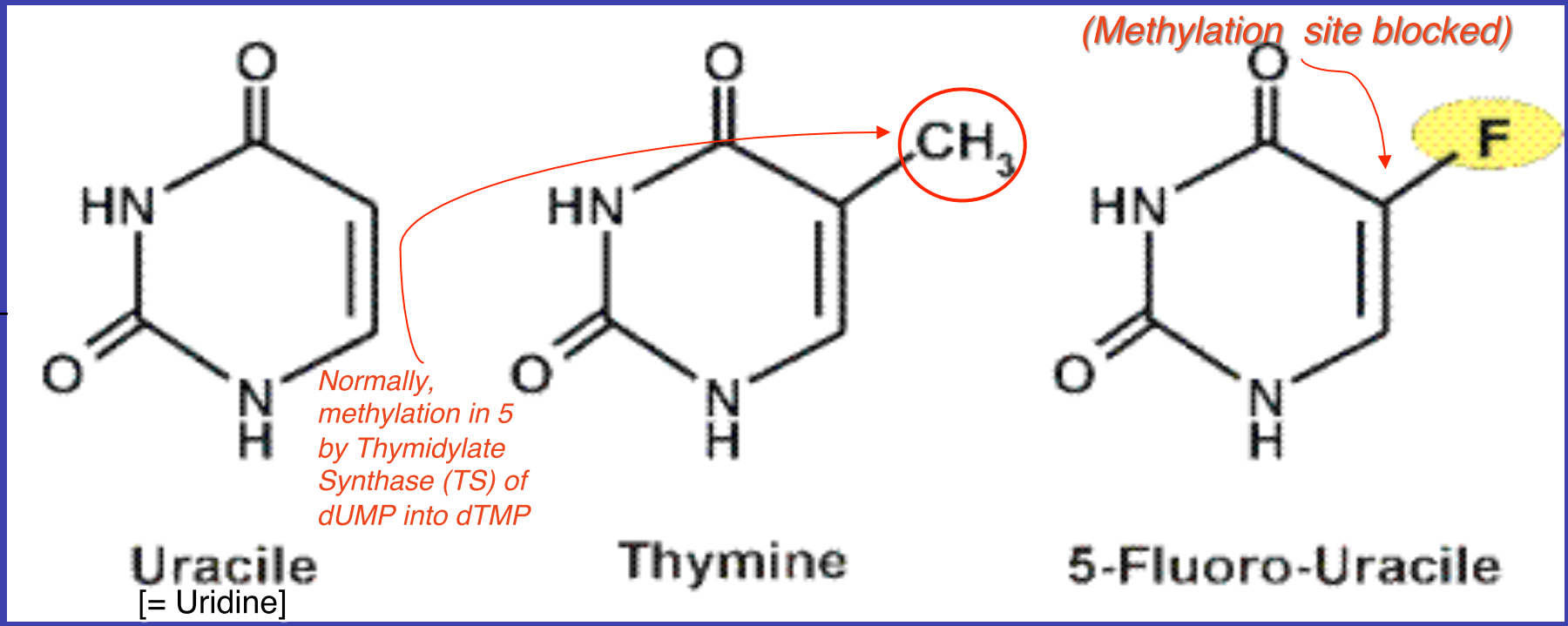
Yet to be studied: p53 dynamics to connect *DNA damage* with *cell cycle arrest*, apoptosis and *repair*



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_ι *and subsequent apoptosis or repair*

(NB: p53 expression is circadian clock-controlled)

2nd example: Drug 5-FU : 50 years on the service of colorectal cancer treatment

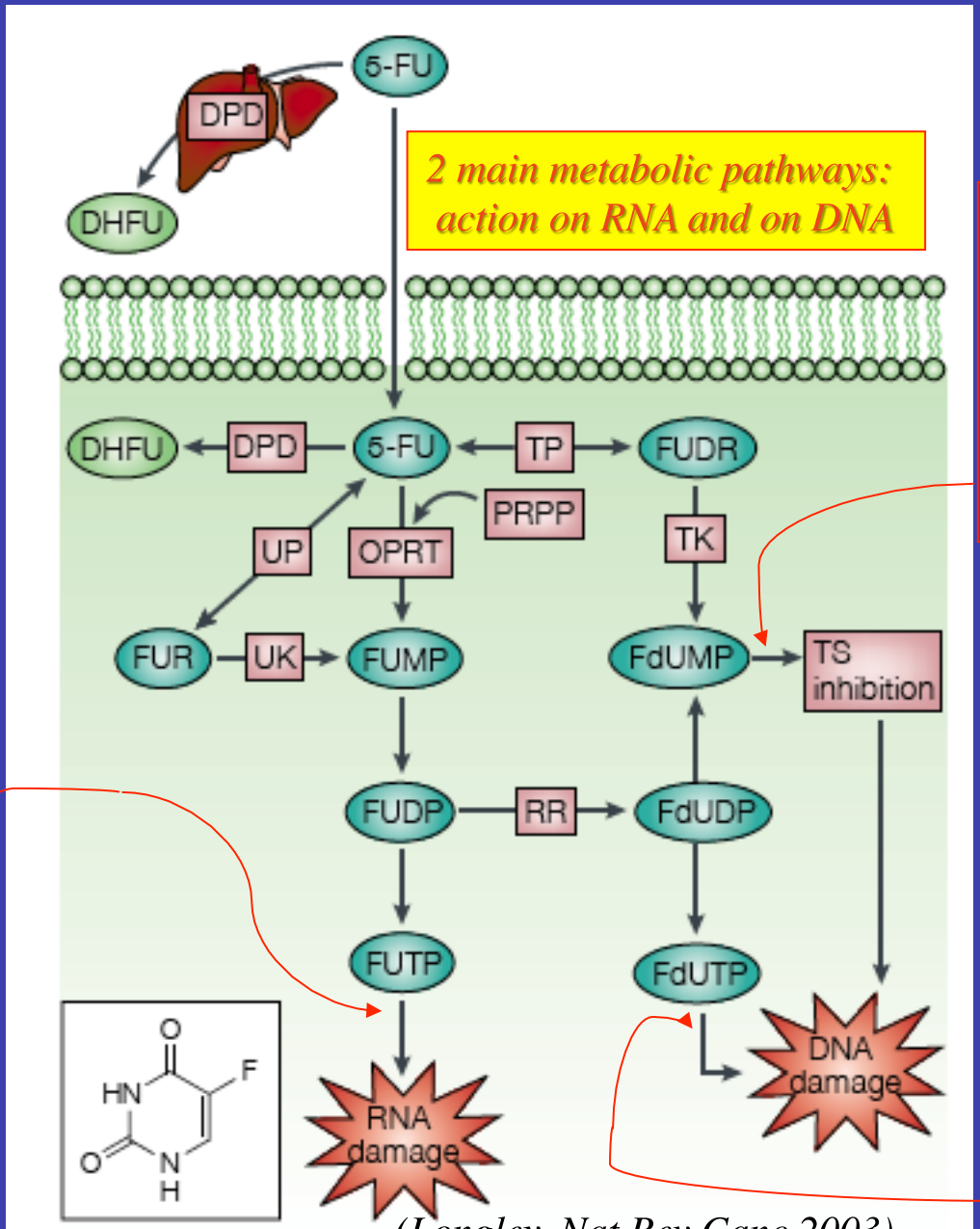


(NB : Uracil is found only in DNA)

Pharmacodynamics (PD) of 5FU

RNA pathway

DNA pathway



Competitive inhibition by FdUMP of dUMP binding to target TS

+

[Stabilisation by CH_2-THF of binary complex dUMP-TS]

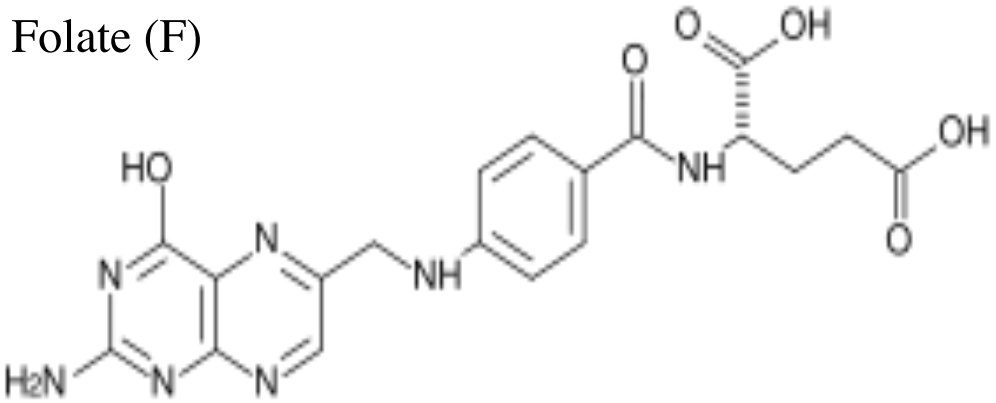
Incorporation of FUTP instead of UTP to RNA

Incorporation of FdUTP instead of dTTP to DNA

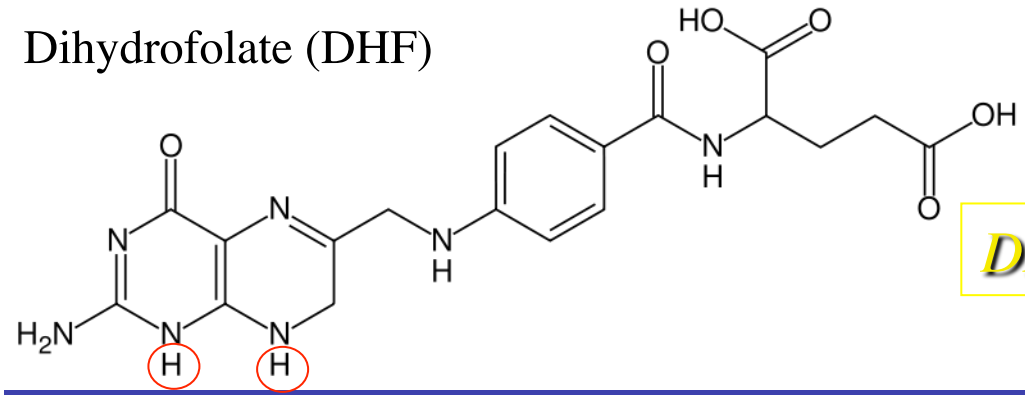
(Longley, Nat Rev Canc 2003)

Folic acid, Leucovorin (LV) and Methylenetetrahydrofolate

Folate (F)

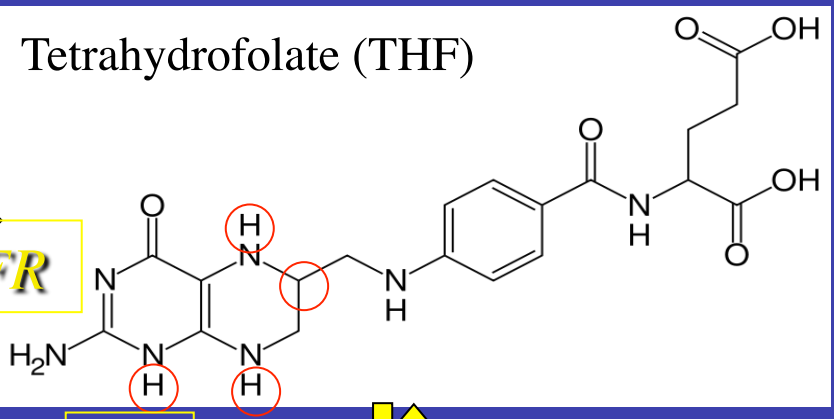


Dihydrofolate (DHF)



DHFR

Tetrahydrofolate (THF)

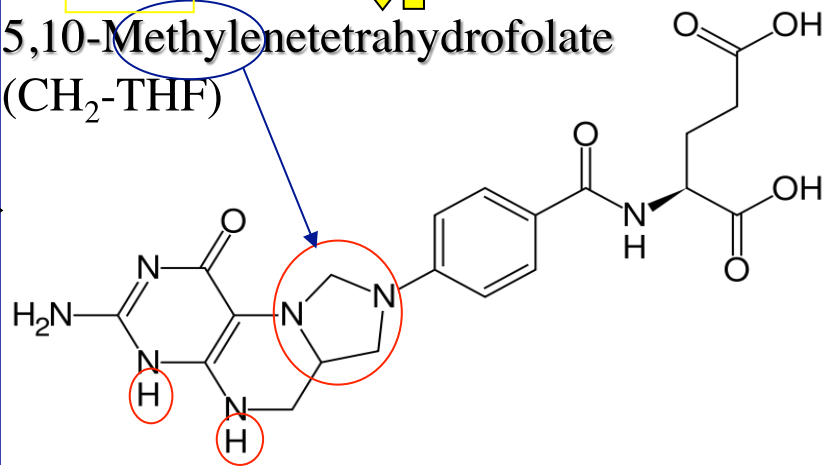


TS

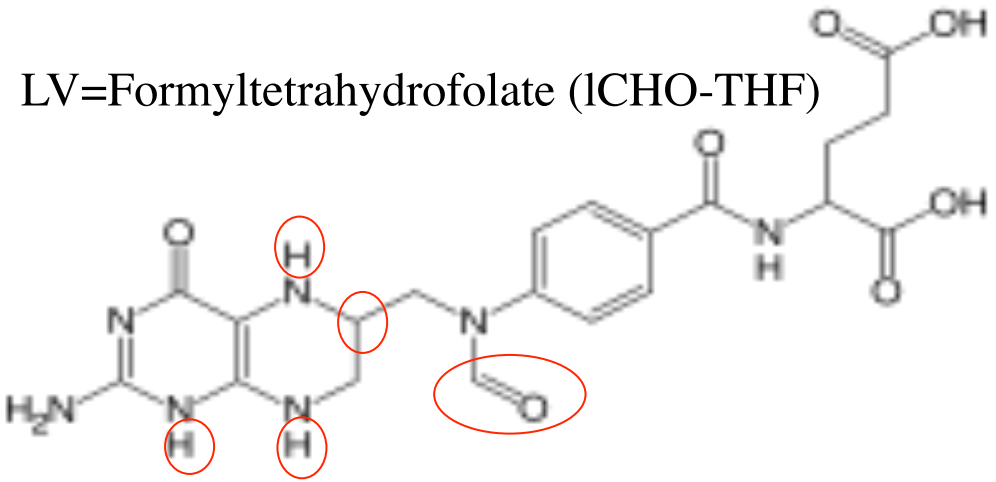
+dTMP

+dUMP

5,10-Methylenetetrahydrofolate (CH_2-THF)



LV=Formyltetrahydrofolate (lCHO-THF)

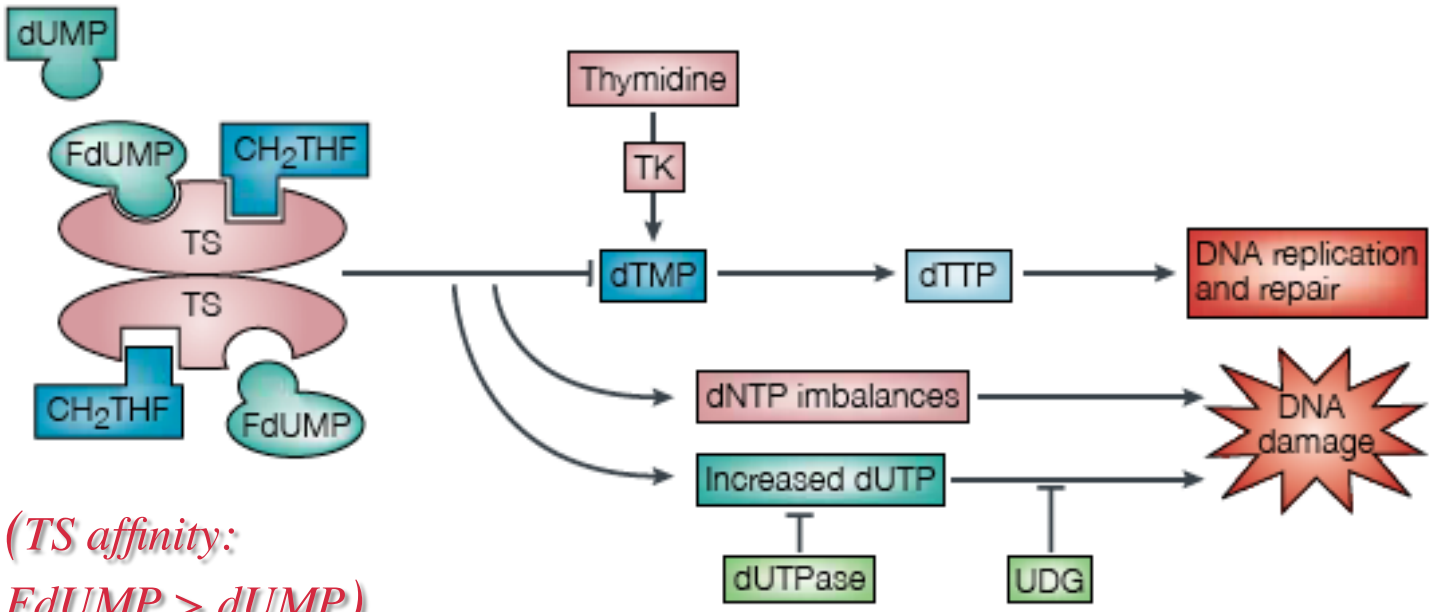


Inhibition of Thymidylate Synthase (TS) by 5FU and Leucovorin

Formyltetrahydrofolate (CHO-THF) = LV a.k.a. Folinic acid, a.k.a. Leucovorin

Precursor of CH_2-THF, coenzyme of TS, that forms with it and FdUMP a *stable ternary complex*, blocking the normal biochemical reaction:

$$5,10\text{-}CH_2THF + dUMP + FADH_2 \xrightleftharpoons{TS} dTMP + THF + FAD$$



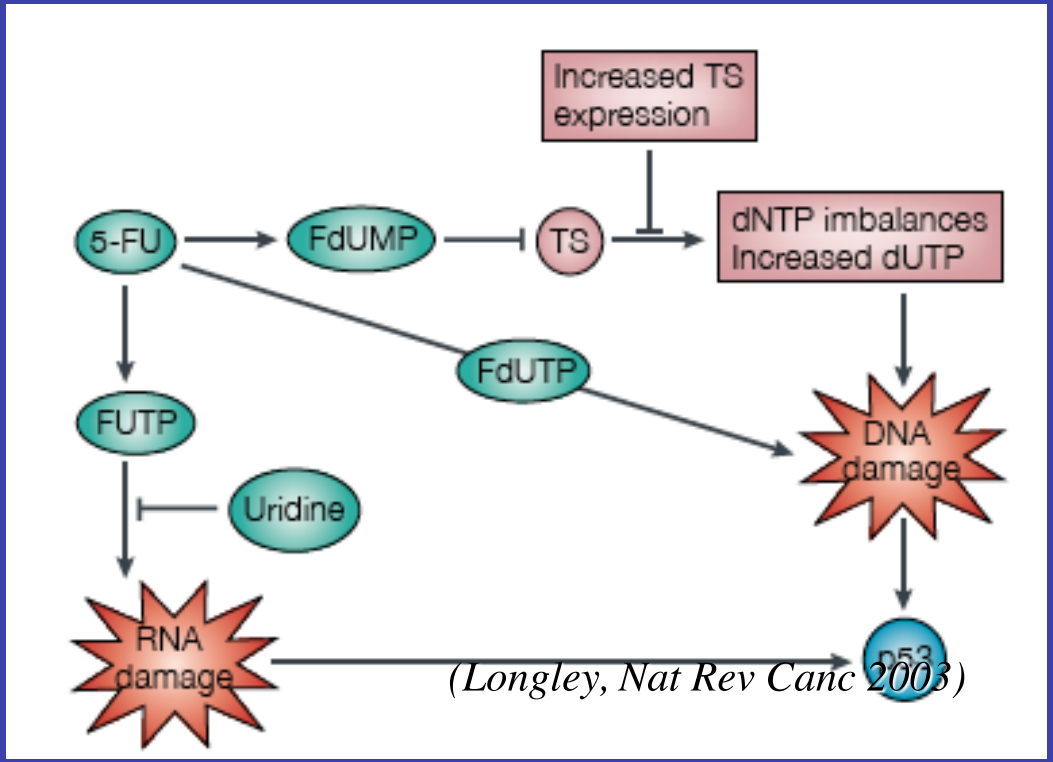
(TS affinity: FdUMP > dUMP)

(Longley, Nat Rev Canc 2003)

Impact on the cell cycle via p53:

1.-junk RNA: by incorporation of FUTP
2.-junk DNA: by incorporation of dUTP and FdUTP
3.-TS blockade: resulting in A/T ratio unbalance

…Hence DNA damage and subsequent triggering of p53



(Longley, Nat Rev Canc 2003)

Plasma and cell pharmacokinetics (PK) of 5FU

- Poor binding to plasma proteins
- Degradation +++ (80%) by liver DPD
- Cell uptake using a un saturable transporter
- Rapid diffusion in fast renewing tissues
- 5-FU = prodrug; main active anabolite = Fd-UMP
- Fd-UMP: active efflux by ABC transporter ABCC11 = MRP8

(Oguri, Mol Canc Therap 2007)

5-FU catabolism: DPD (dihydropyrimidine dehydrogenase)

- 5-FU $\xrightarrow{\text{DPD}}$ 5-FU H_2, hydrolysable [$\rightarrow$ FβAlanin]
- DPD: hepatic +++
- DPD: limiting enzyme of 5FU catabolism
- Michaelian kinetics
- Circadian rhythm of activity
- Genetic polymorphism +++ (very variable toxicity)

Modelling PK-PD of 5FU (+ drug resistance) + Leucovorin

$$(1)\quad \frac{dP}{dt} = -k_0 P - \frac{aP}{b+P} - l_{DPD}\frac{P}{m_{DPD}+P} + \frac{i(t)}{V}$$

$$(2)\quad \frac{dF}{dt} = \frac{a}{\xi}\frac{P}{b+P} - \frac{AF}{c+F} - k_1 FS + k_{-1}B$$

$$(3)\quad \frac{dQ}{dt} = -k_2 Q + \frac{j(t)}{V}$$

$$(4)\quad \frac{dL}{dt} = \frac{k_2}{\xi}Q - k_3 L - k_4 BL$$

$$(5)\quad \frac{dN}{dt} = \frac{\kappa F^n}{\lambda^n + F^n} - \mu N$$

$$(6)\quad \frac{dA}{dt} = \mu N - \nu A$$

$$(7)\quad \frac{dS}{dt} = -k_1 FS + k_{-1}B + \theta_{TS}(S_0 - S)$$

$$(8)\quad \frac{dB}{dt} = k_1 FS - k_{-1}B - k_4 BL$$

$$(9)\quad \frac{dT}{dt} = k_4 BL - v_T T$$

Input j = LV infusion flow

Input i = 5-FU infusion flow

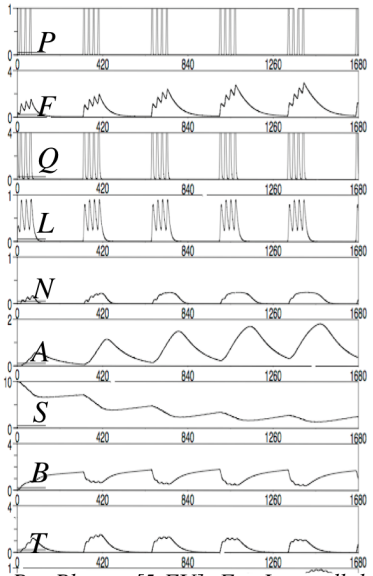
A = ABC transporter (active drug efflux)

S = Free Thymidylate Synthase (TS)

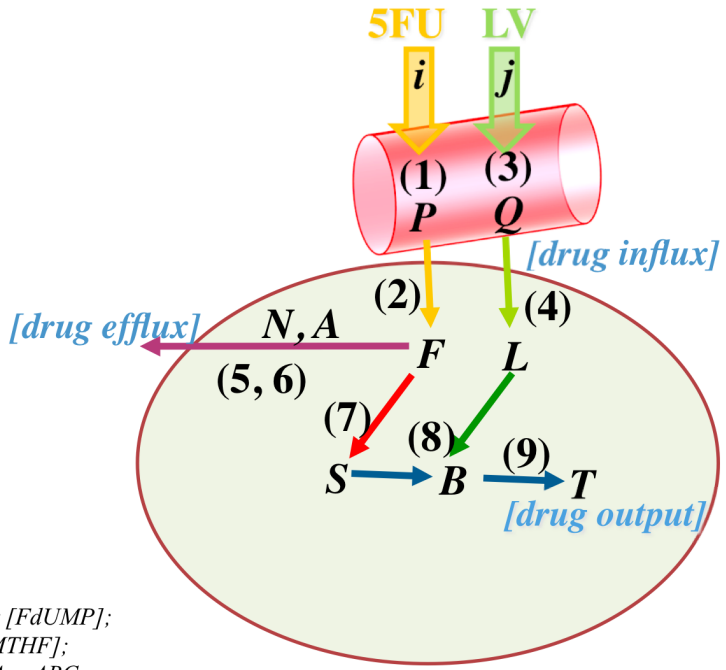
Drug output T = Blocked Thymidylate Synthase (stable ternary FdUMP-MTHF-TS complex)

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

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



P = Plasma [5-FU]; F = Intracellular [FdUMP];
Q = Plasma [LV]; L = Intracellular [MTHF];
N = 5-FU-triggered Nuclear Factor; A = ABC Transporter activity, NuclearFactor-induced;
S = Free [TS] (not FdUMP-bound);
B = [FdUMP-TS] reversible binary complex;
T = [FdUMP-TS-MTHF] stable ternary complex



(Lévi, Okyar, Dulong, Innominato,, JC., Annu Rev Pharmacol Toxicol 2010)

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 series 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 4FU+LV infusion,12 hours a day, every other week

P = Plasma [5FU]

F = Intracellular [FdUMP]

Q = Plasma [LV]

L = Intracellular [LV]

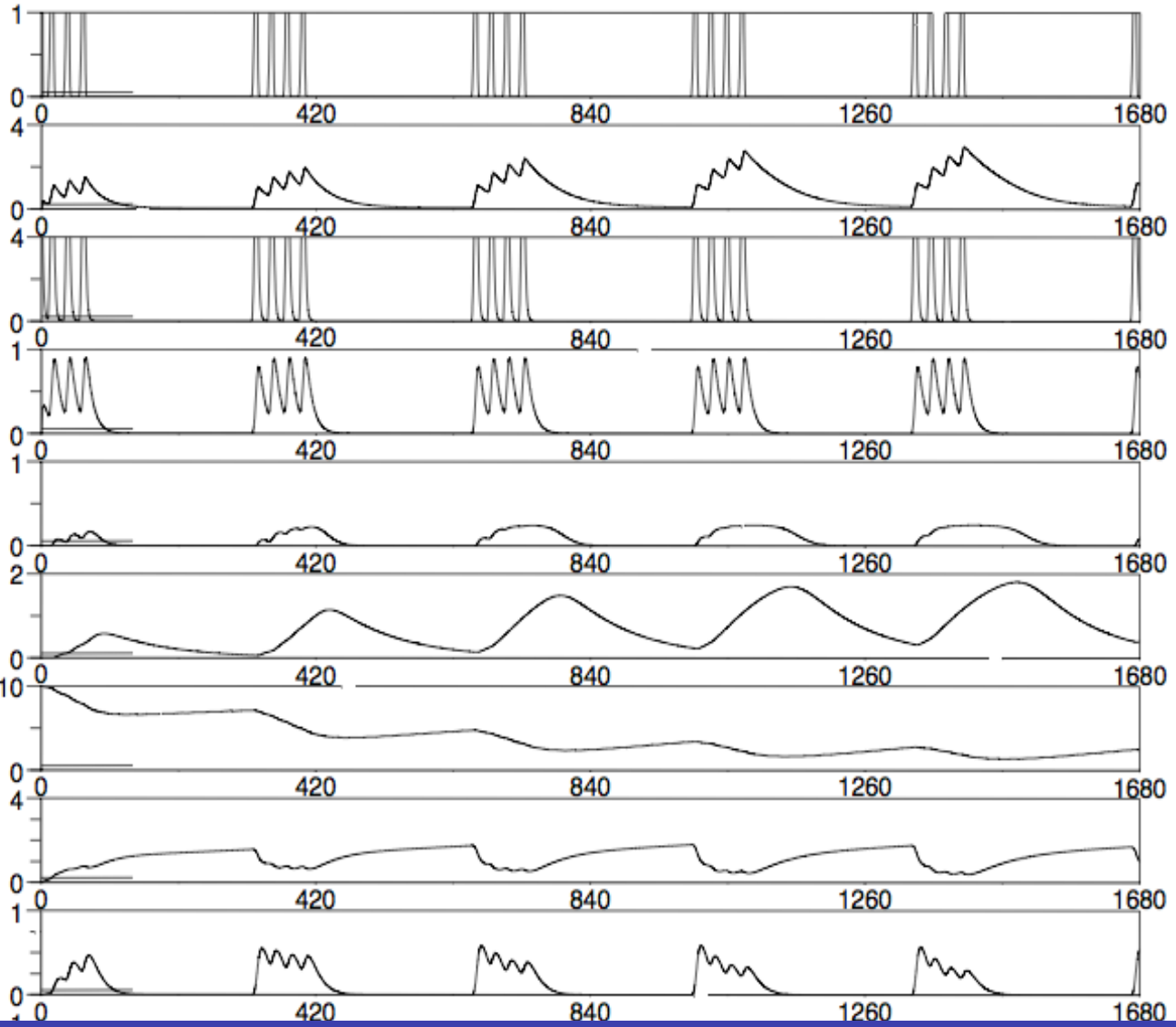
N = [nrf2] 5FU-triggered Nuclear Factor

A = ABC Transporter activity, nrf2-inducted

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

B = [FdUMP-TS] reversible binary complex

T = [FdUMP-TS-LV] stable ternary complex



5FU and LV: plasma and intracellular PK

FdUMP extracellular efflux (by ABC Transporter ABCC11)

5FU cell uptake

5FU DPD detoxication in liver

$i(t)$ = 5FU infusion flow

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

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

$j(t)$ = LV infusion flow

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

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

Binding of FdUMP to TS to form a *reversible* binary complex B

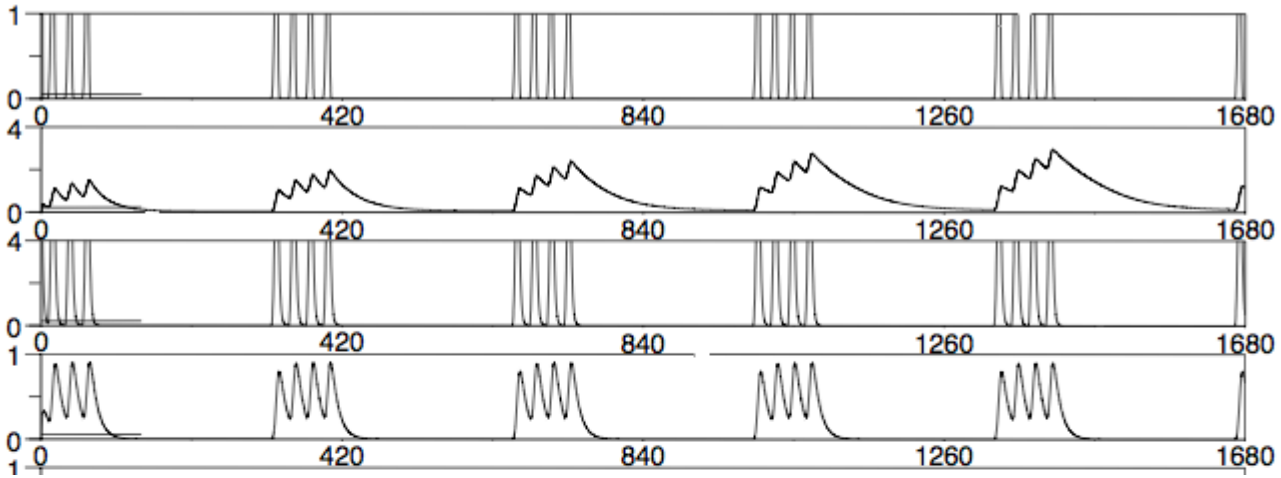
Binding of LV to FdUMP-TS = B to form a *stable* ternary complex

P=5FU (plasma)

F=FdUMP (cell)

Q=LV (plasma)

L=LV (cell)



Assuming induction of ABC Transporter activity by FdUMP-triggered synthesis of a nuclear factor [*nrf2?*]

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

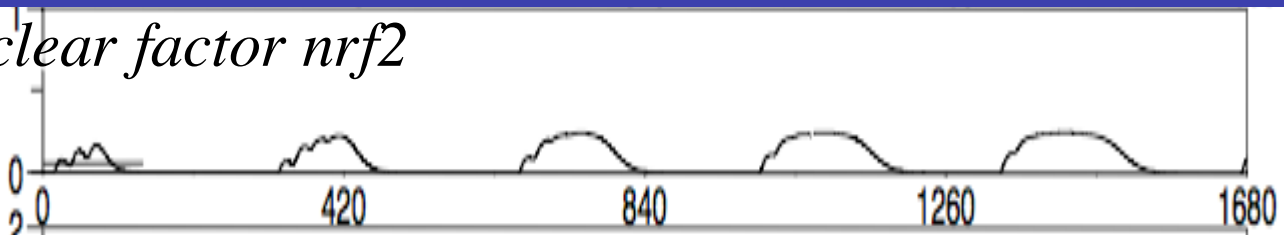
$$\frac{dN}{dt} = \frac{\kappa F^n}{\lambda^n + F^n} - \mu N$$

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

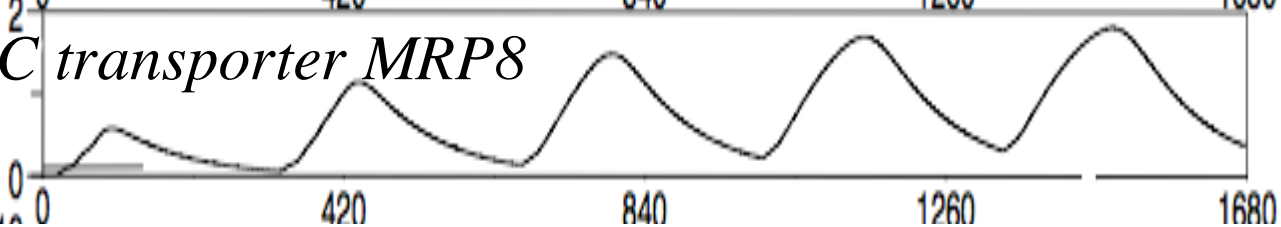
Nuclear factor

ABC Transporter (ABCC11=MRP8)

N=nuclear factor nrf2



A=ABC transporter MRP8

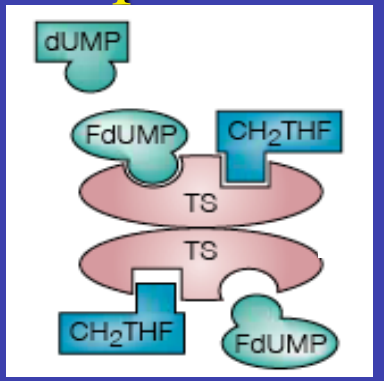


Targeting Thimidylate Synthase (*TS*) by FdUMP: Formation of binary and ternary *TS*-complexes

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

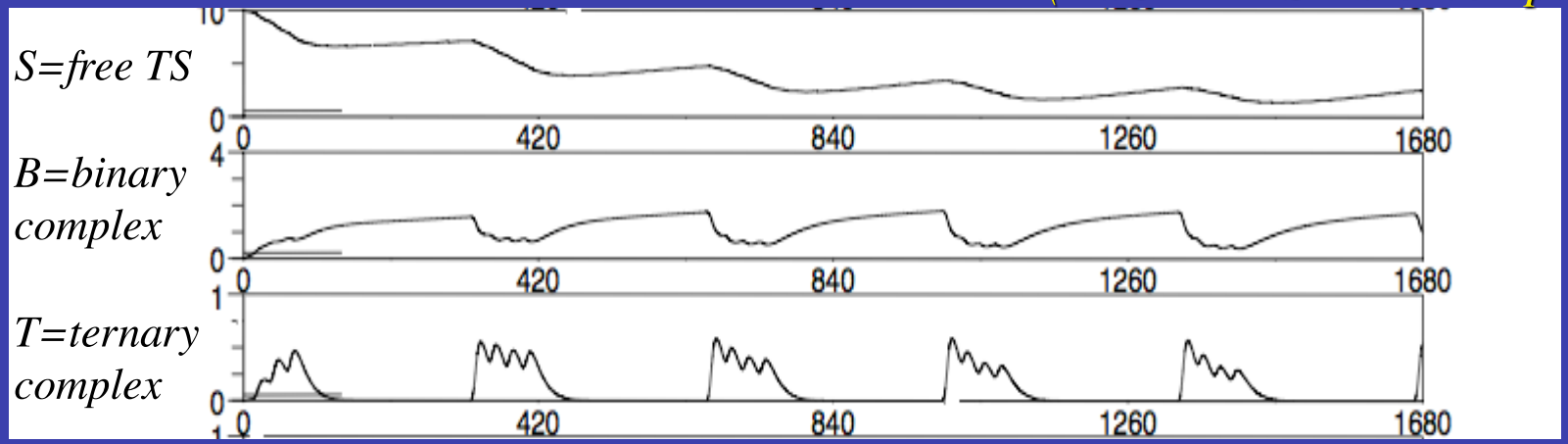
$$\frac{dB}{dt} = k_1 FS - k_{-1} B - k_4 BL$$

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



$F + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} F\text{-}S = B$ (*FdUMP-TS 2-complex*)

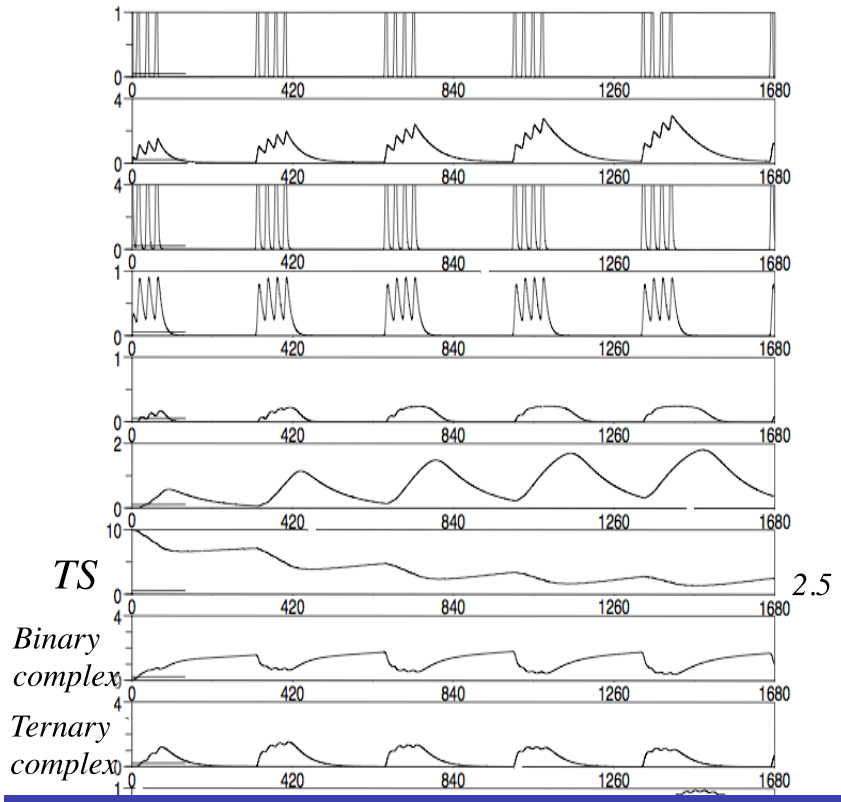
$B + L \xrightarrow{k_4} B\text{-}L = T$ (*FdUMP-TS-LV 3-complex*)



Some features of the model:

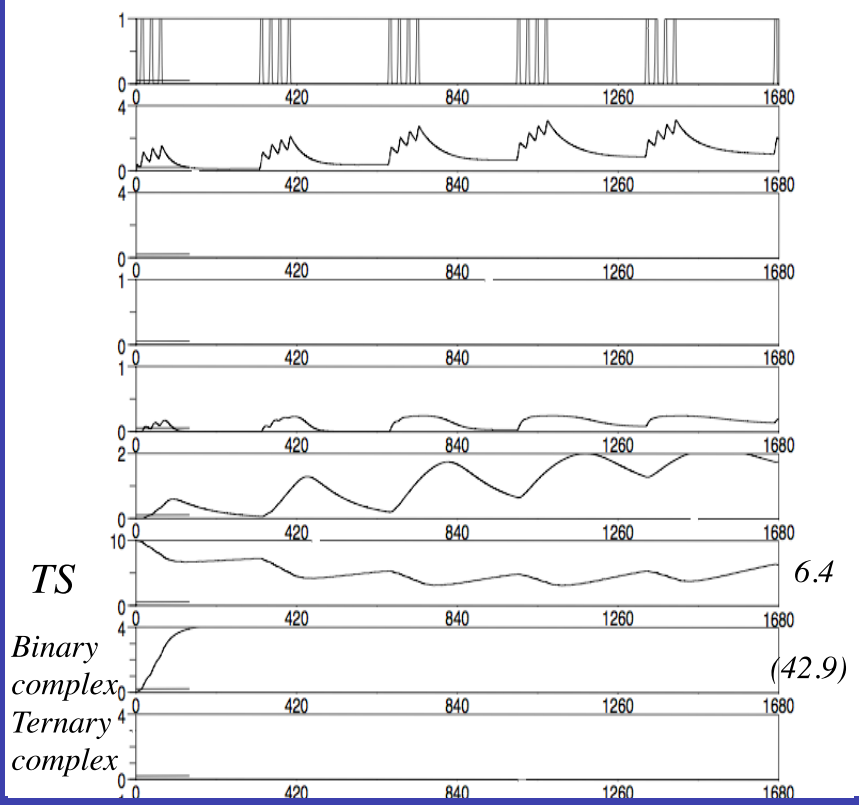
a) 5FU with/without LV in cancer cells (=MRP8+)

With Leucovorin added in treatment



Cancer cells die

Without Leucovorin added

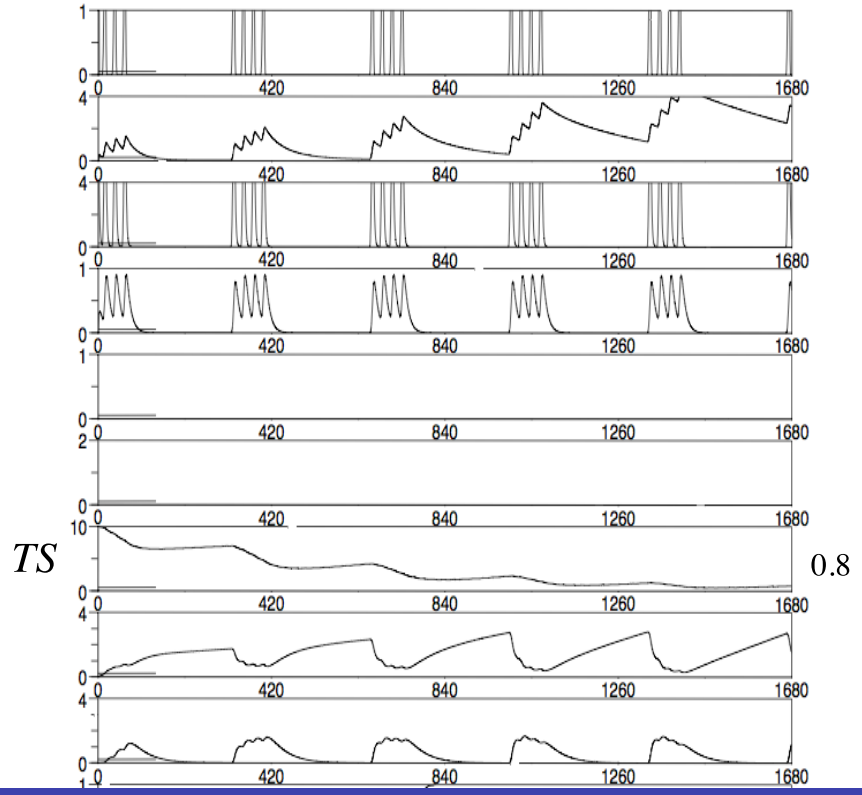


Cancer cells survive

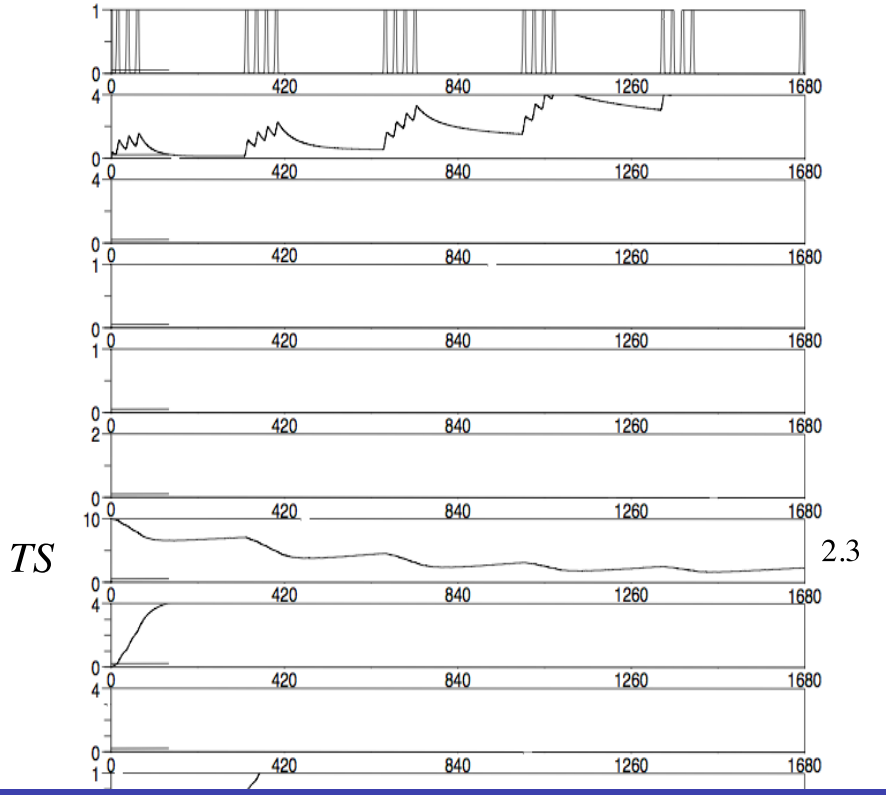
b) 5FU with/without LV in healthy cells (=MRP8-)

…But adding LV also kills more healthy cells:

With Leucovorin added in treatment

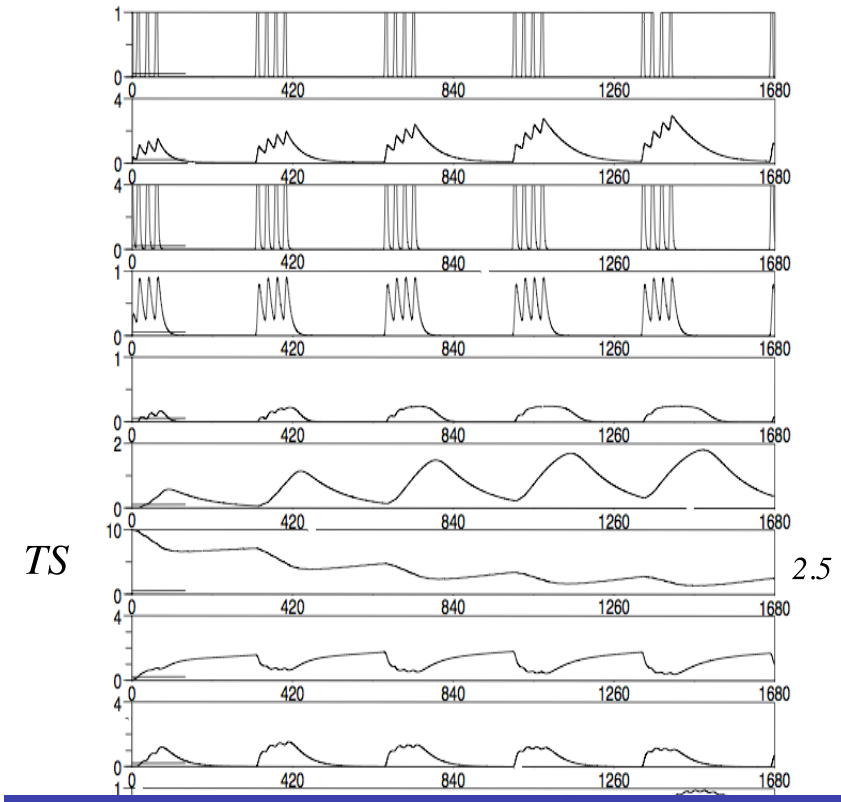


Without Leucovorin added

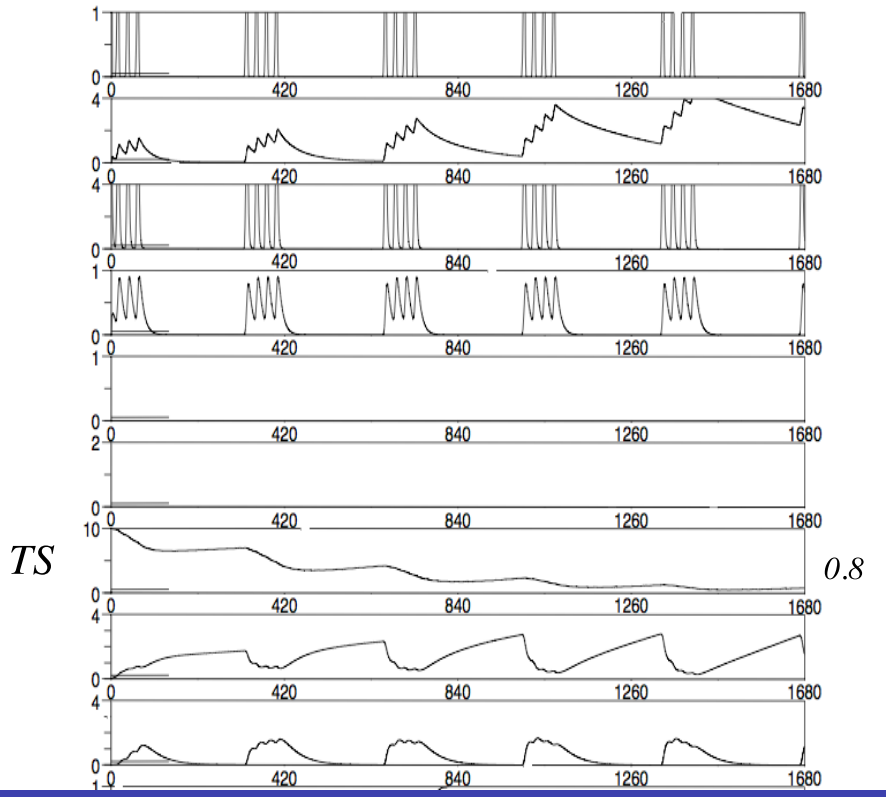


c) 5FU+LV with/without MRP8 (cancer vs. healthy cells)

Cancer cells (=MRP8+)



Healthy cells (=MRP8-)

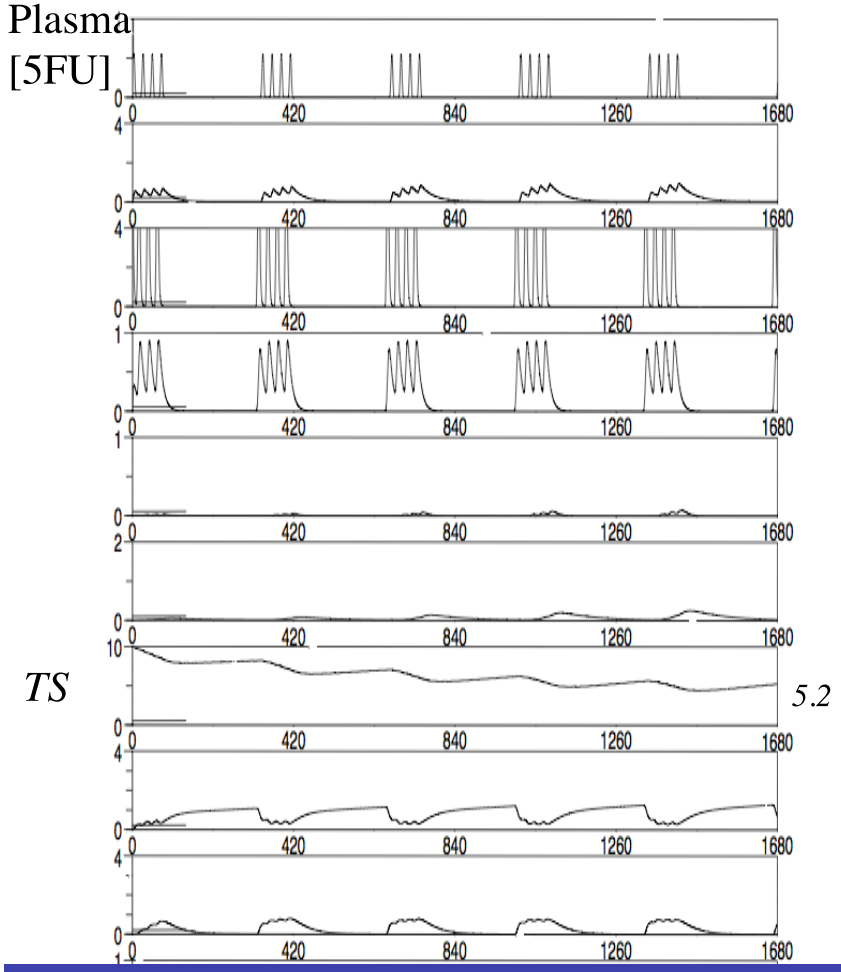


Cancer cells resist more than healthy cells, due to lesser exposure to FdUMP

(actively effluxed from cells by ABC Transporter MRP8)

d) 5FU+LV with chronotherapeutics: Infusion phase differences in cancer cells (MRP8+)

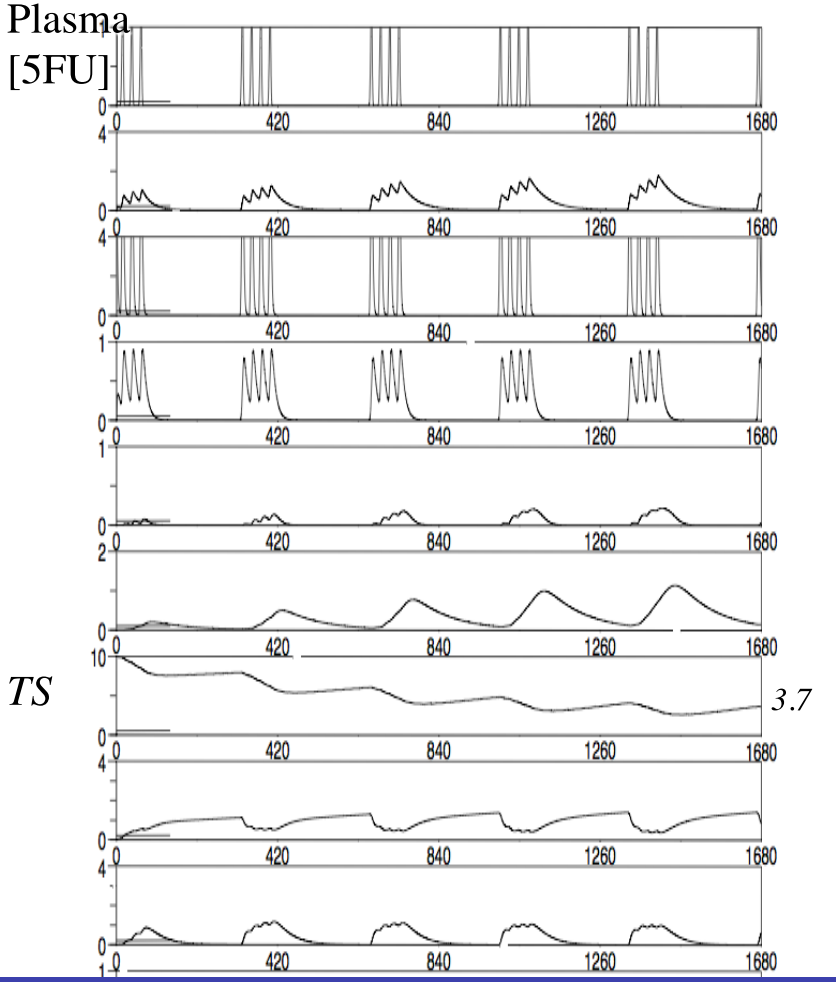
DPD and 5FU in phase



Cancer cells die

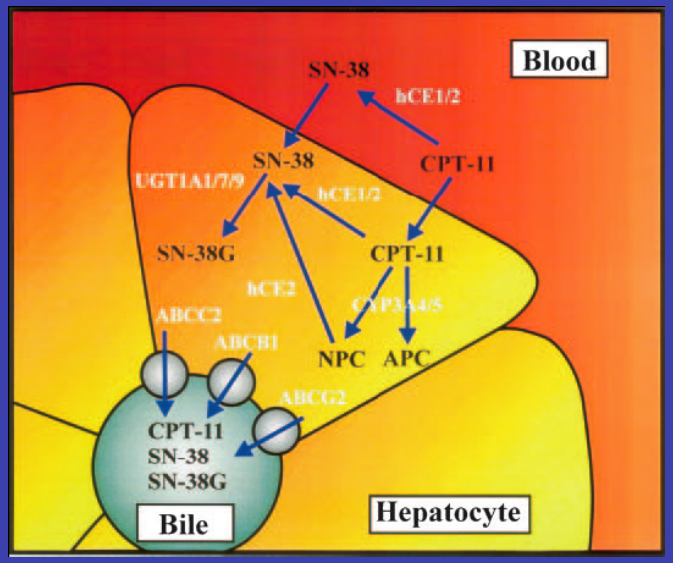
[The same behaviour can be shown in healthy cells]

DPD and 5FU out of phase



Cancer cells die even more (more 5FU in plasma, more FdUMP in cells)

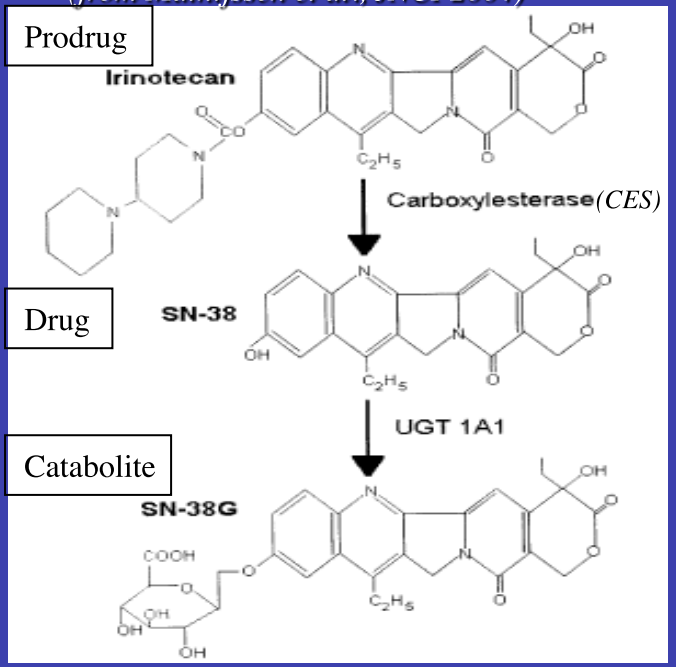
3rd example: Drug *Irinotecan* (CPT11)



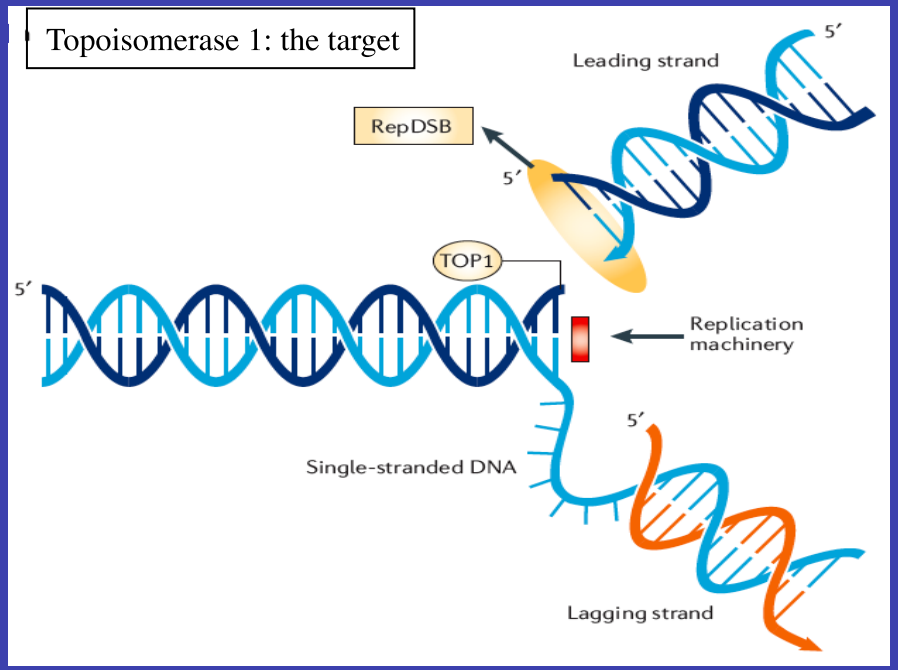
(from Mathijssen et al., JNCI 2004)

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

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

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

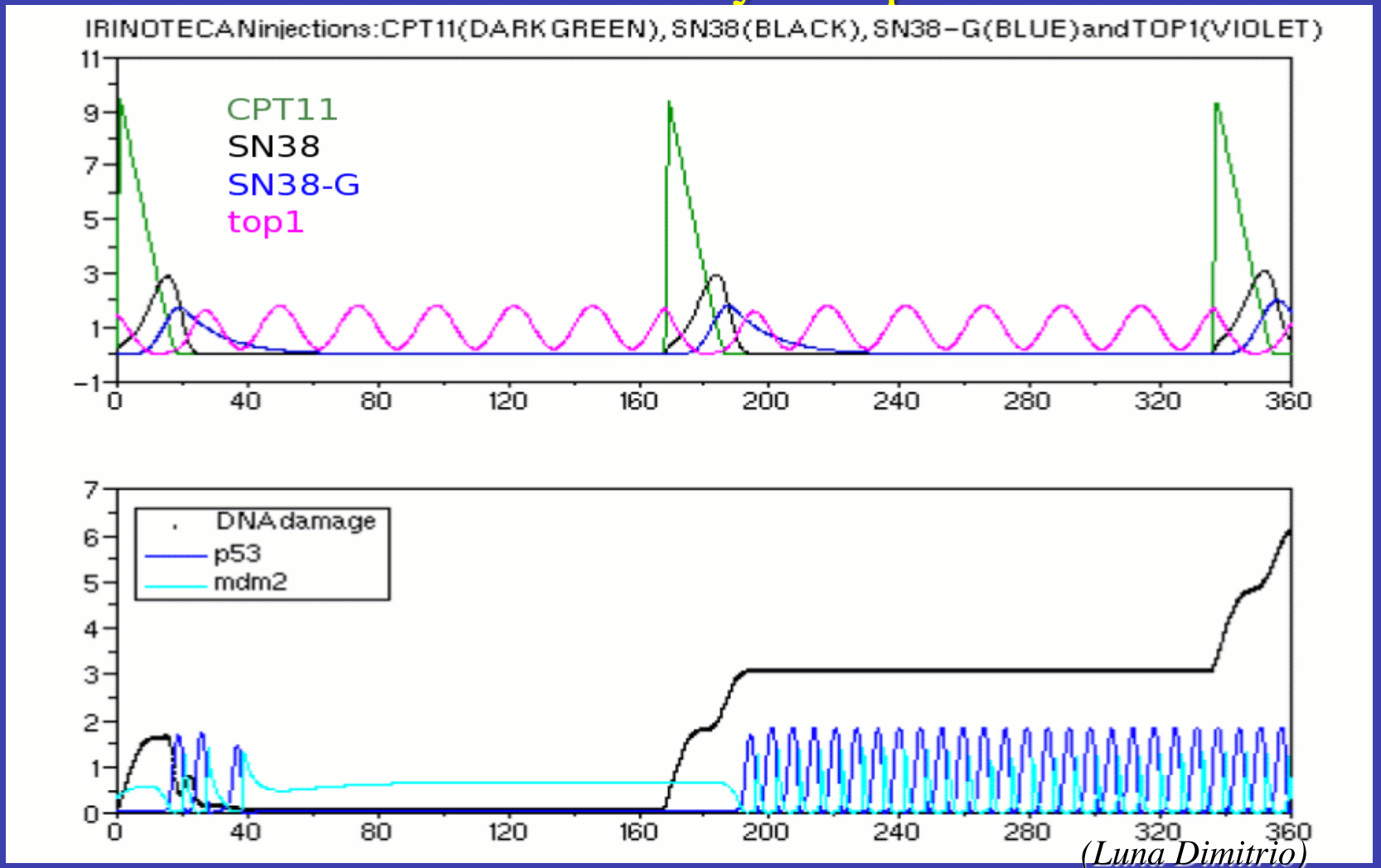
(Luna Dimitrio's Master thesis 2007)

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)

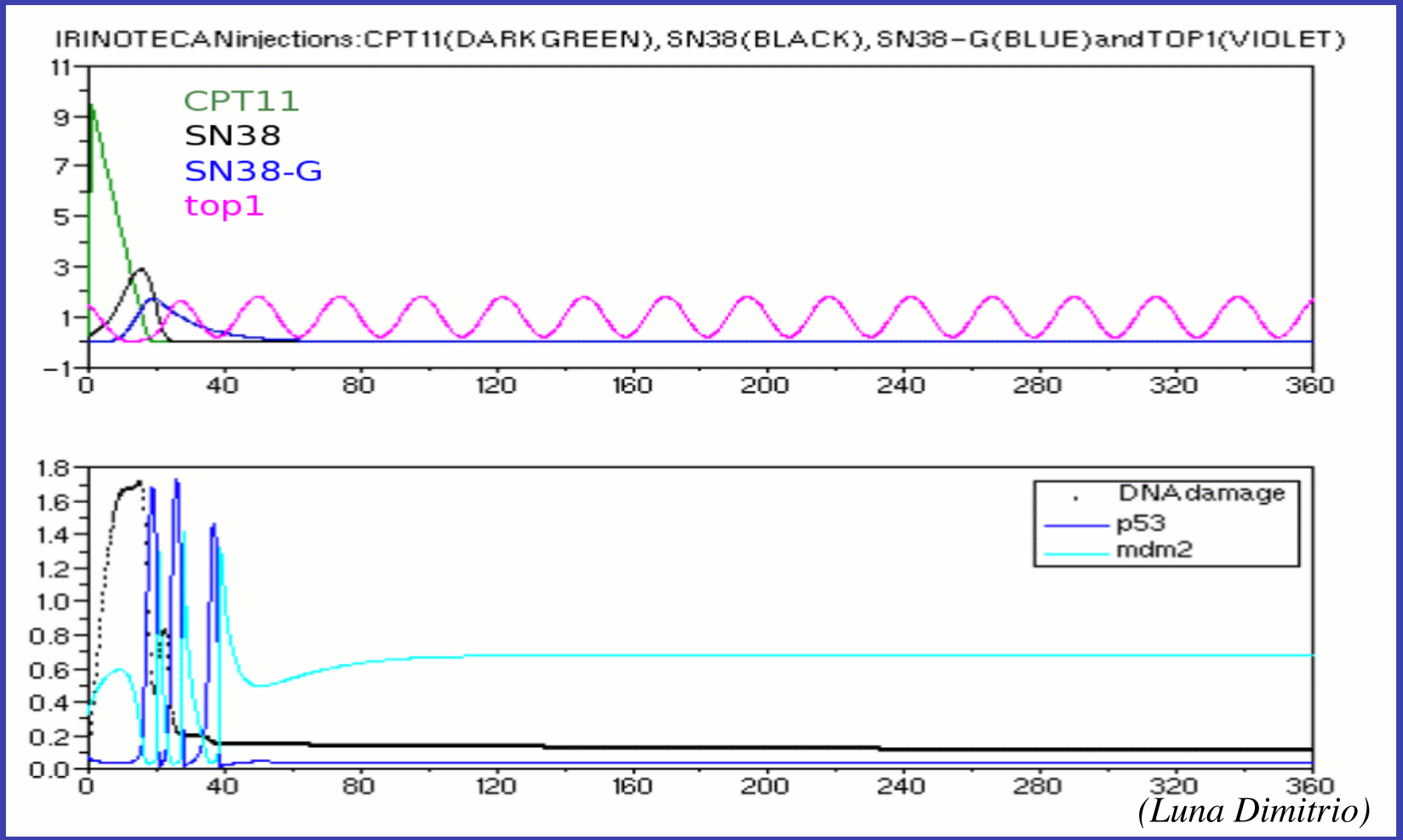
PD of *Irinotecan*: 1) p53-MDM2 oscillations can repair DNA damage provided that not too much SN38-TOP1-DNA ternary complex accumulates



(Luna Dimitrio)

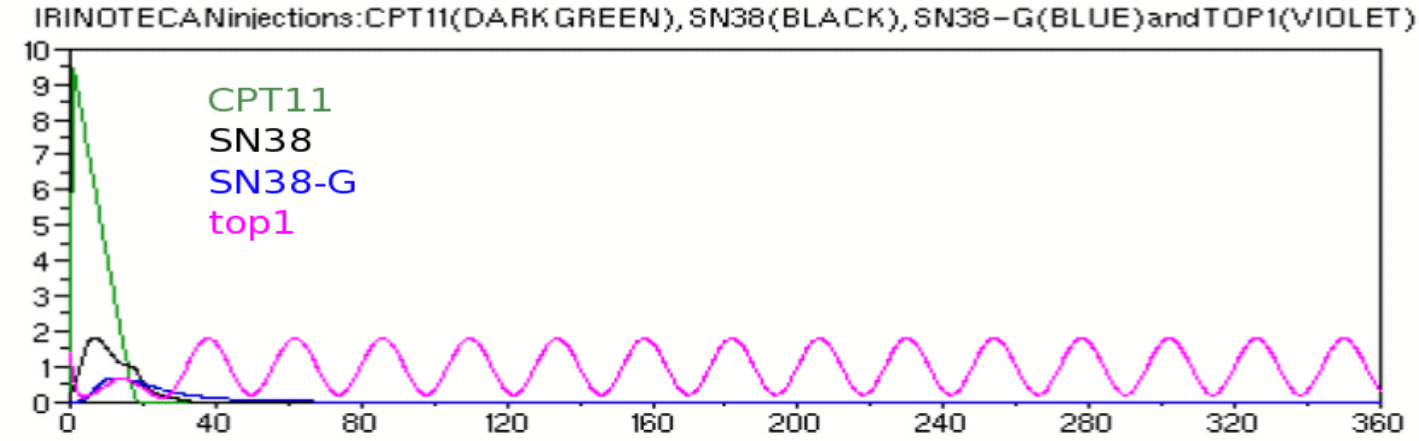
(Intracellular PK-PD of irinotecan and A. Ciliberto's model of p53-MDM2 oscillations)

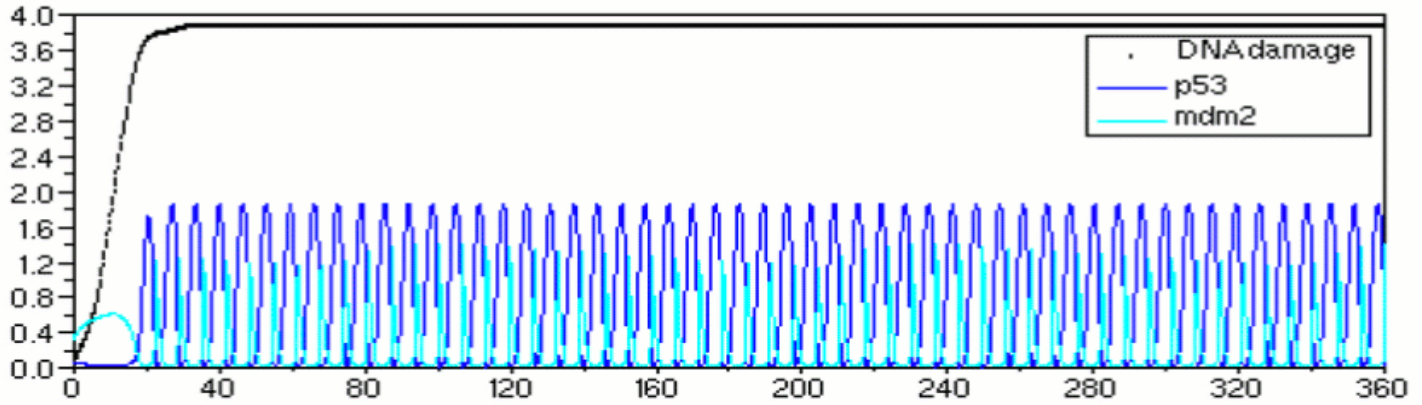
2) A single infusion of *Irinotecan*, *out of phase* with TOP1 circadian rhythm, creates *reversible damages*: DNA damage is repaired after a few oscillations of p53



(Luna Dimitrio)

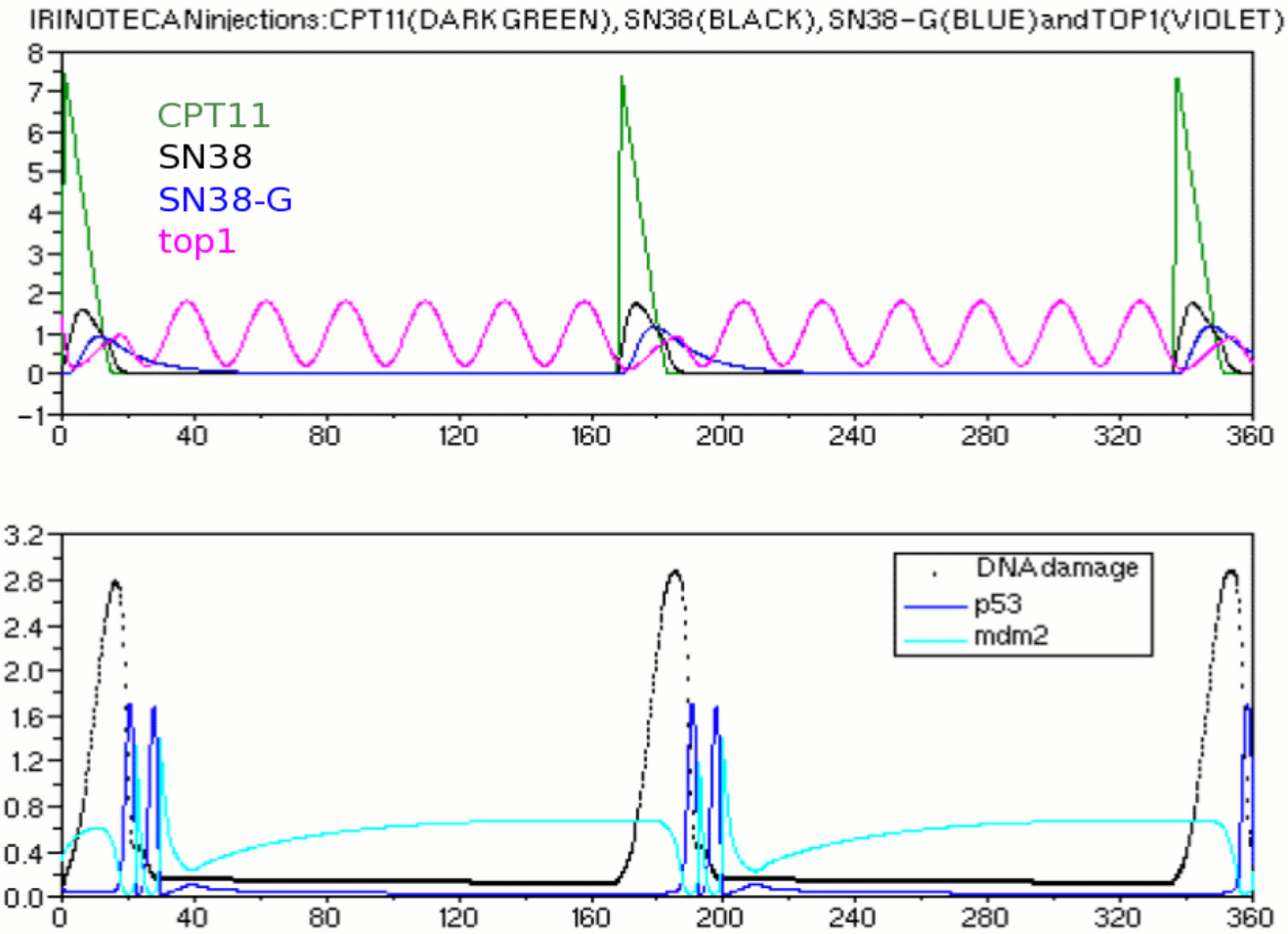
3) A single infusion of *Irinotecan*, *in phase* with TOP1 circadian rhythm, creates *irreversible damages*: p53 oscillations cannot repair the damage to DNA



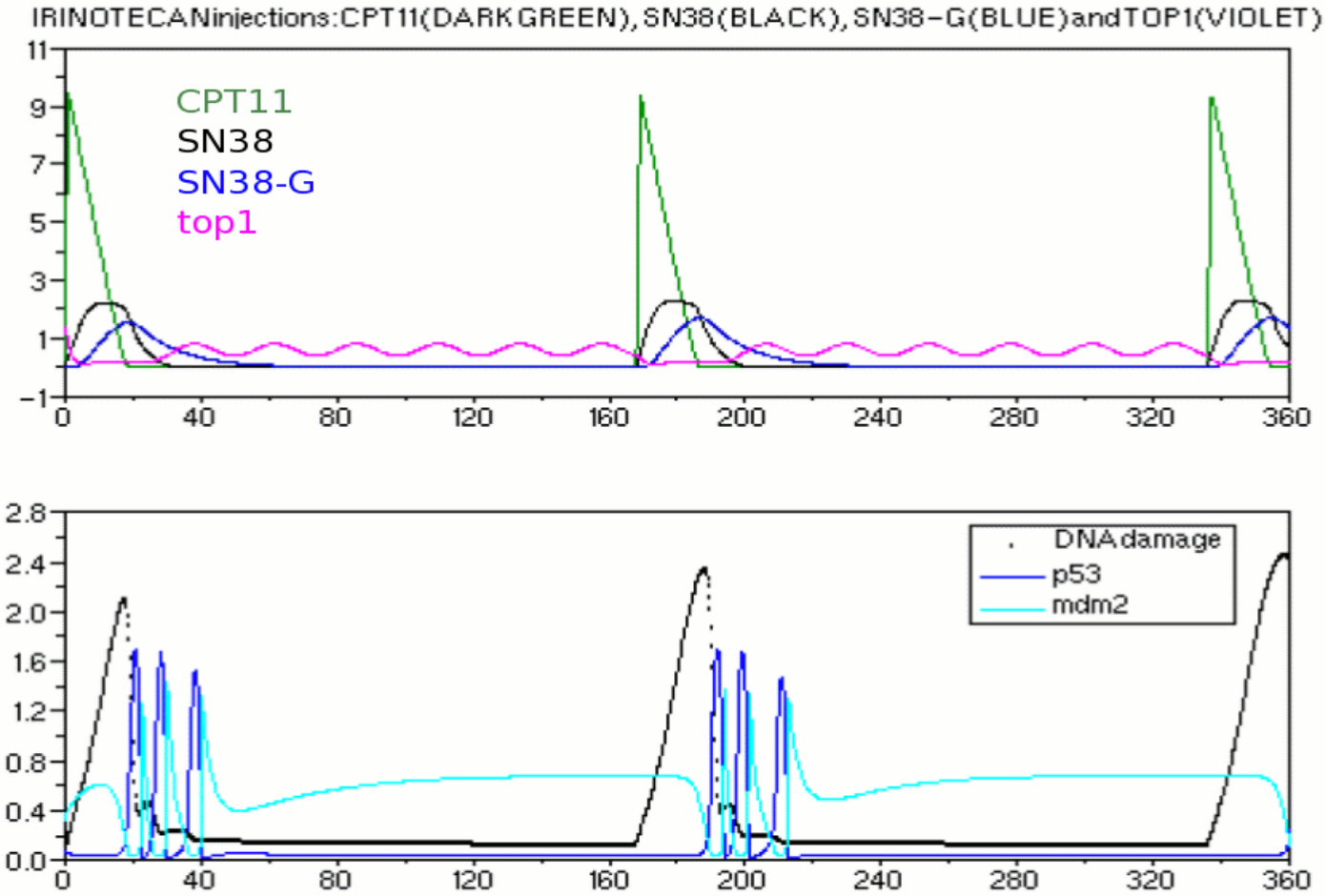


(Luna Dimitrio)

4) Doubled activity of degrading enzyme UGT1A1 [known way of resistance to CPT11]: 3 infusions do not kill the cell



5) Lower production and weaker periodic forcing =downregulation of TOP 1 [another way of resistance to CPT11]: DNA damage is repaired

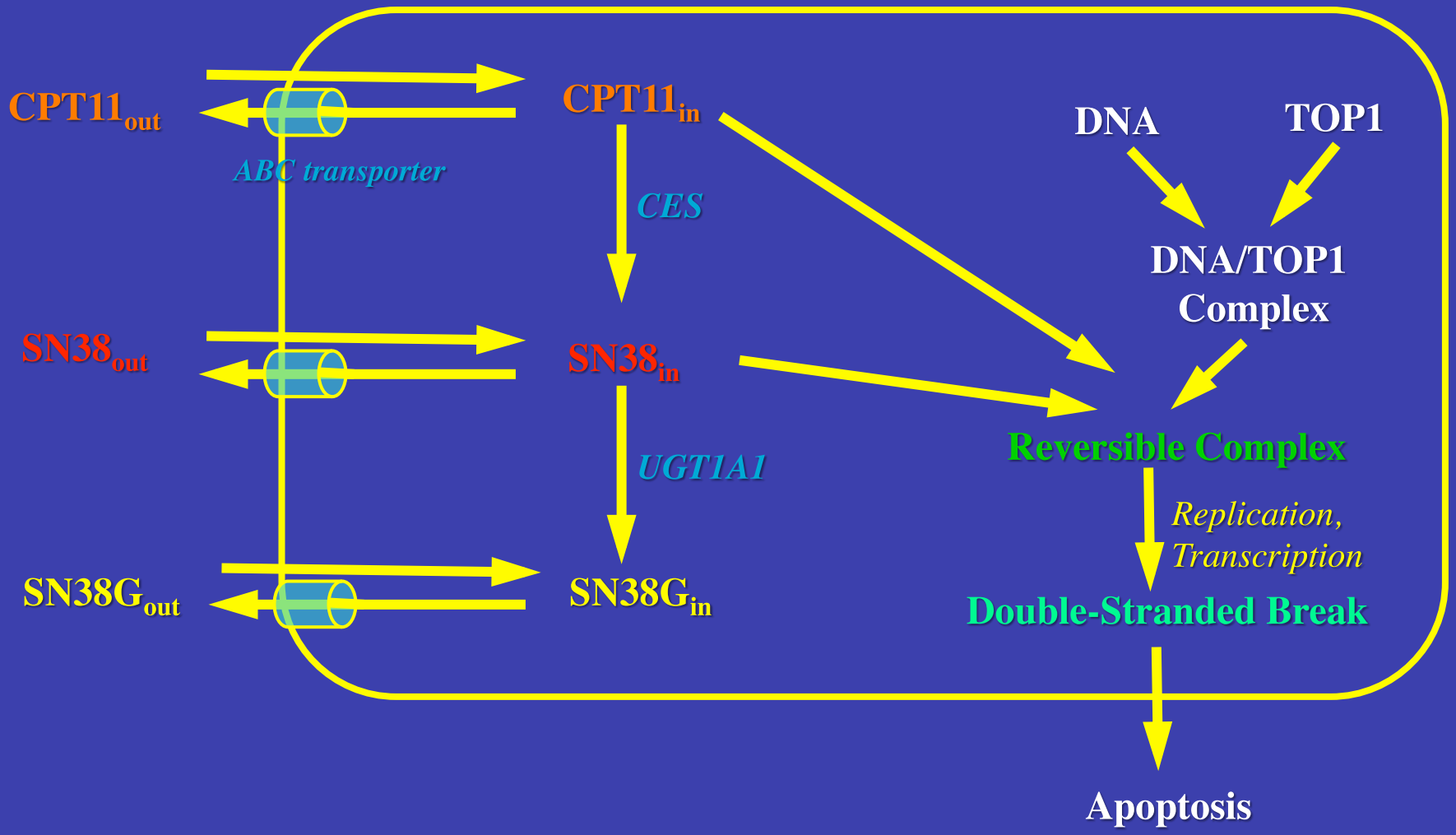


More on Irinotecan: experimental identification of model parameters in *nonproliferative* cell cultures

(from Annabelle Ballesta's PhD work)

- No interaction with the cell cycle: confluent populations of CaCo2 cells
- Pharmacodynamics: measurement of DNA double strand breaks
- Circadian clocks synchronised by seric shock (fetal bovine serum)
- Activation / degradation enzyme expression, concentration and activity
- Transmembrane exchanges by ABC transporters (active efflux pumps)

What must be in the PK-PD model



+Impact of circadian clocks

Mathematical Modelling

PK-PD model: 8 ODEs, 18 parameters

$$\frac{d[CPT11_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{uptCPT}\frac{V_{out}}{V_{in}}[CPT11_{out}] + \frac{V_{effCPT}[ABCB1][CPT11_{in}]}{K_{effCPT}+[CPT11_{in}]} \quad (1)$$

$$\frac{d[CPT11_{in}]}{dt} = k_{uptCPT}\frac{V_{out}}{V_{in}}[CPT11_{out}] - \frac{V_{effCPT}[ABCB1][CPT11_{in}]}{K_{effCPT}+[CPT11_{in}]} - \frac{V_{CPT-SN}[CPT11_{in}]}{K_{CPT-SN}+[CPT11_{in}]} \quad (2)$$

$$\frac{d[SN38_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{uptSN}\frac{V_{out}}{V_{in}}[SN38_{out}] + \frac{V_{effSN}[ABCG2][SN38_{in}]}{K_{effSN}+[SN38_{in}]} \quad (3)$$

$$\frac{d[SN38_{in}]}{dt} = k_{uptSN}\frac{V_{out}}{V_{in}}[SN38_{out}] - \frac{V_{effSN}[ABCG2][SN38_{in}]}{K_{effSN}+[SN38_{in}]} + \frac{V_{CPT-SN}[CPT11_{in}]}{K_{CPT-SN}+[CPT11_{in}]}$$
$$-\frac{V_{glu}[UGT][SN38_{in}]}{K_{glu}+[SN38_{in}]} - k_{fC}[TOP1][SN38_{in}](DNA_{tot}-[COMPL]) + k_{rC}[COMPL] \quad (4)$$

$$\frac{d[SN38G_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{uptSNG}\frac{V_{out}}{V_{in}}[SN38G_{out}] + \frac{V_{effG}[ABCG2][SN38G_{in}]}{K_{effSN}+[SN38G_{in}]} \quad (5)$$

$$\frac{d[SN38G_{in}]}{dt} = k_{uptSNG}\frac{V_{out}}{V_{in}}[SN38G_{out}] - \frac{V_{effG}[ABCG2][SN38G_{in}]}{K_{effG}+[SN38G_{in}]} + \frac{V_{glu}[UGT][SN38_{in}]}{K_{glu}+[SN38_{in}]} \quad (6)$$

$$\frac{d[COMPL]}{dt} = k_{fC}[TOP1][SN38_{in}](DNA_{tot}-[COMPL]-[DSB]) - k_{rC}[COMPL] - k_{DSB}[COMPL] \quad (7)$$

$$\frac{d[DSB]}{dt} = k_{DSB}[COMPL] \quad (8)$$

(A.Ballesta et al., article in preparation)

Mathematical modelling

Zoom on equation for [$CPT11_{out}$]:

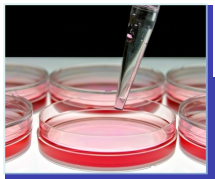
$$\frac{d[CPT11_{out}]}{dt}\frac{V_{out}}{V_{in}} = -k_{uptakeCPT}\frac{V_{out}}{V_{in}}[CPT11_{out}] + \frac{V_{effCPT}[ABC][CPT11_{in}]}{K_{effCPT}+[CPT11_{in}]}$$

Change over time

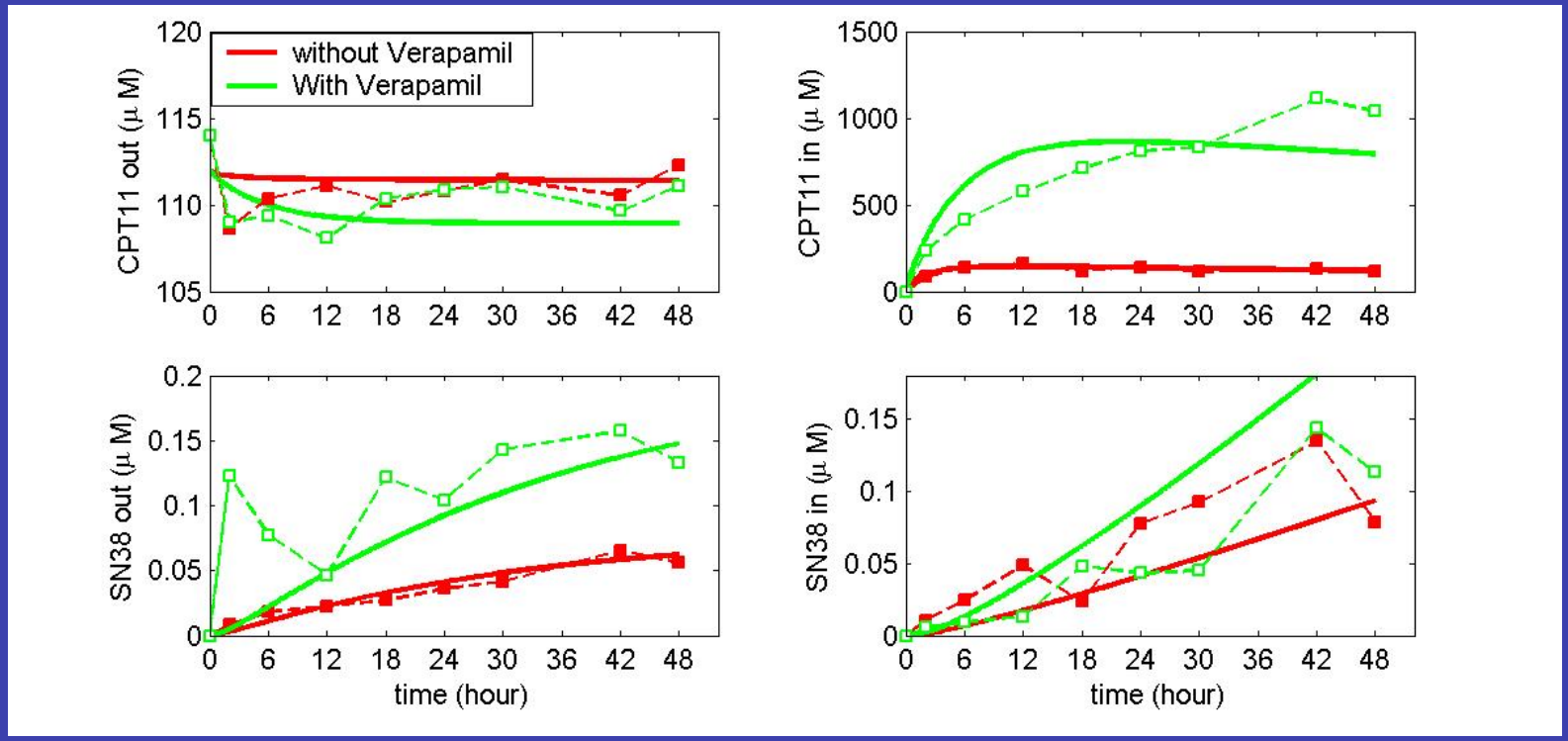
CPT11 cell uptake (passive)

CPT11 cell efflux (active= Michaelis-Menten kinetics)

- [$CPT11_{out}$] = CPT11 extracellular concentration
- [$CPT11_{in}$] = CPT11 intracellular concentration
- V_{out} = volume of extracellular medium
- V_{in} =volume of intracellular medium
- $k_{uptakeCPT}$= speed of CPT11 uptake
- V_{effCPT},K_{eff} =Michaelis Menten parameters for CPT11 efflux

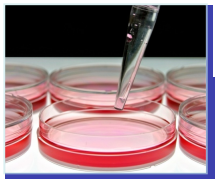


Experimental results on Caco2 cells: kinetic study



Exposure of Caco2 cells to CPT11 (115µM) during 48H, pre-incubated or not with Verapamil 100 µM (inhibitor of ABCB1), measurement of [CPT11] and [SN38] by HPLC

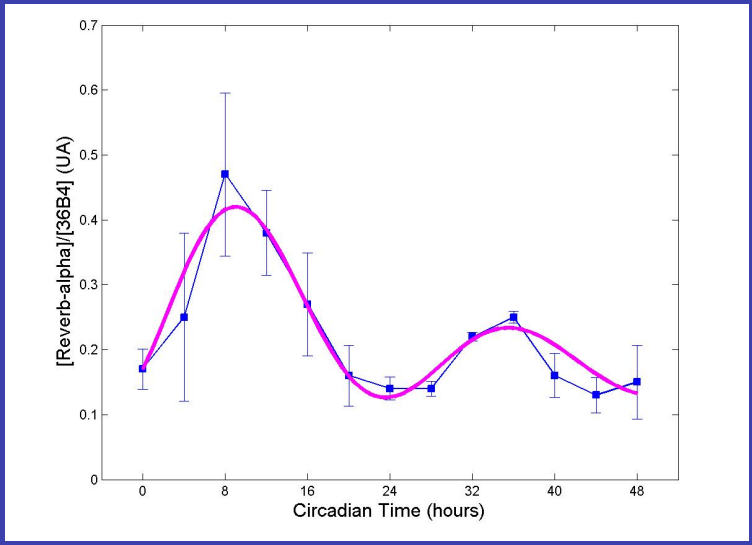
- CPT11 Bioactivation into SN38
- ABCB1 involved in CPT11 efflux but not in SN38 efflux



Experimental results on Caco-2 cells: circadian clocks

- Seric shocks (ie. exposing cells to a large amount of nutrients during 2 hours) synchronise the circadian clock of the cells which subsequently oscillate in synchrony

- Three clock genes (RevErb-α, Per2, Bmal1) oscillate in Caco-2 cells -> circadian clocks work properly

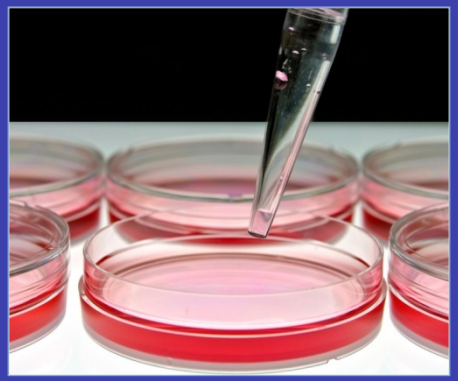


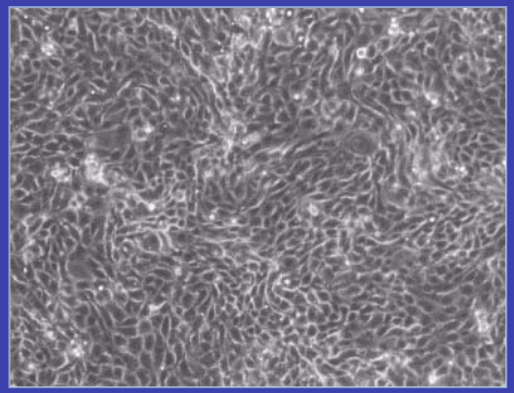
mRNA Curve Fitting:

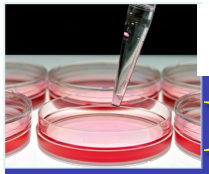
$$[mRNA](t) = R + Se^{\lambda t}(1 + \epsilon cos(\frac{2\pi}{T} + \phi))$$

mRNA measurement by quantitative RT-PCR

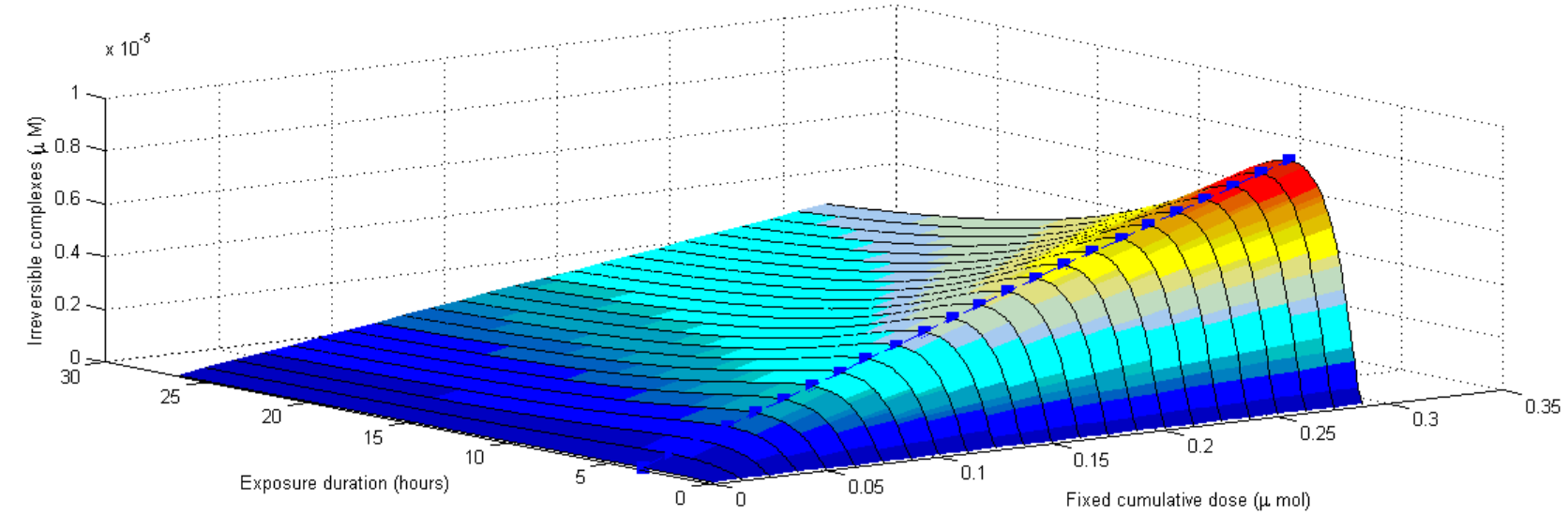
Optimising exposure to Irinotecan in CaCo2 cell cultures

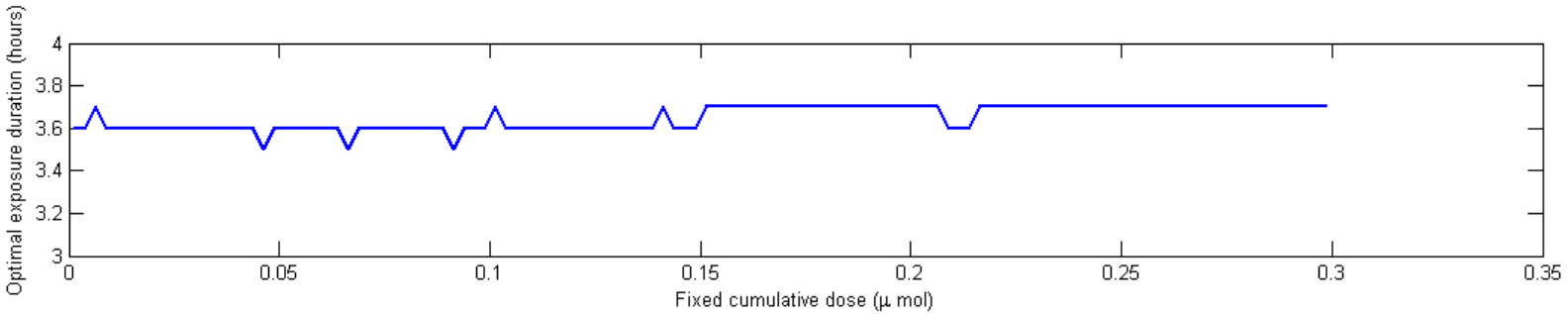




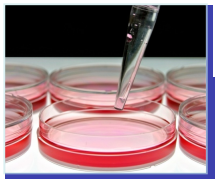


Irinotecan exposure optimisation in nonsynchronised cells (assumed to represent cancer cells)

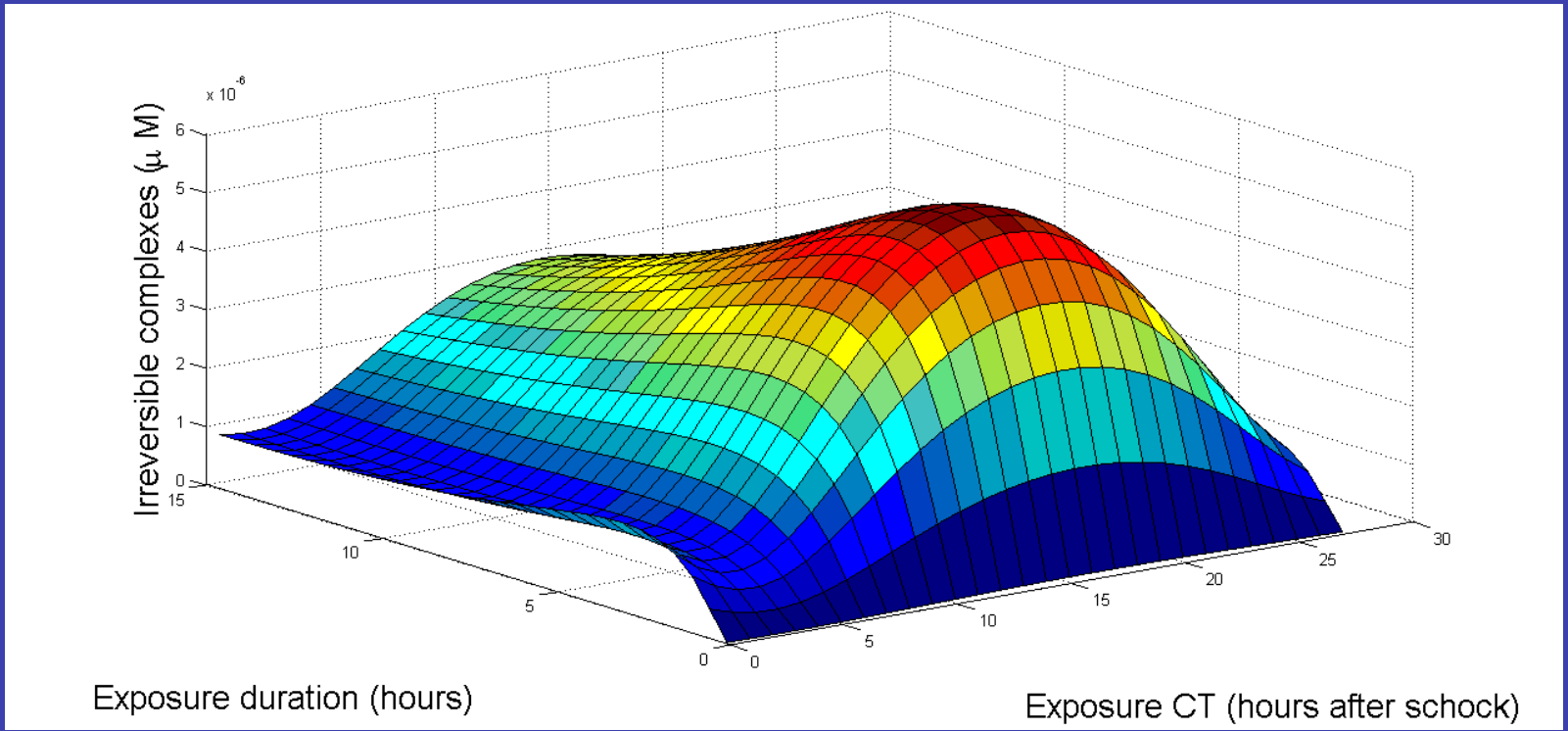




For a fixed cumulative dose of Irinotecan, *optimal exposure duration of 3.6 hours*, independently of the dose

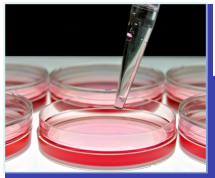


Irinotecan exposure optimisation for synchronised cells (assumed to represent healthy cells)

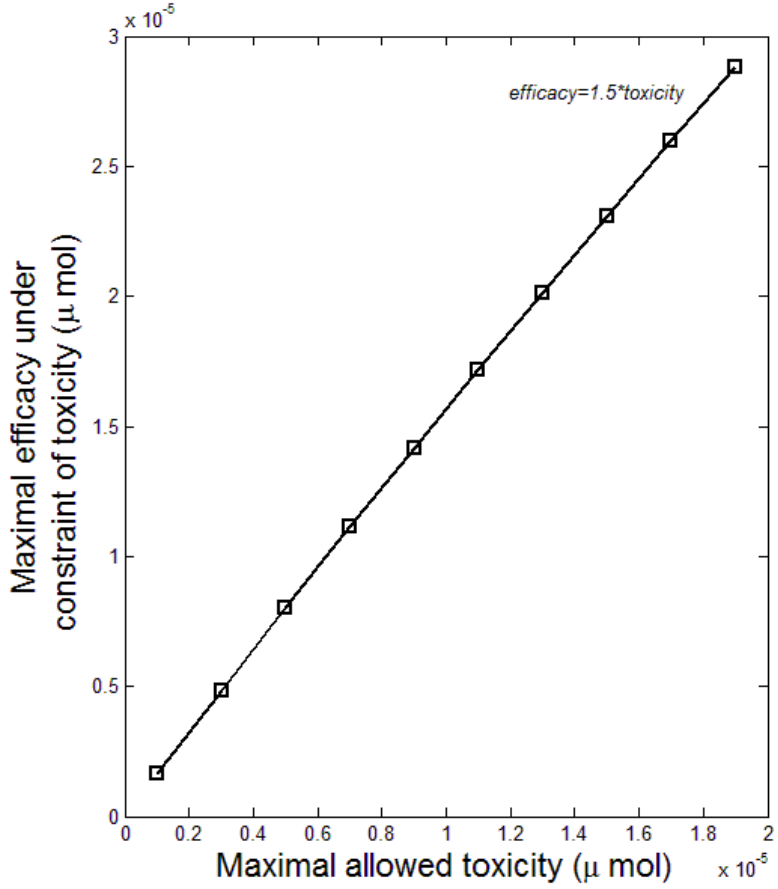


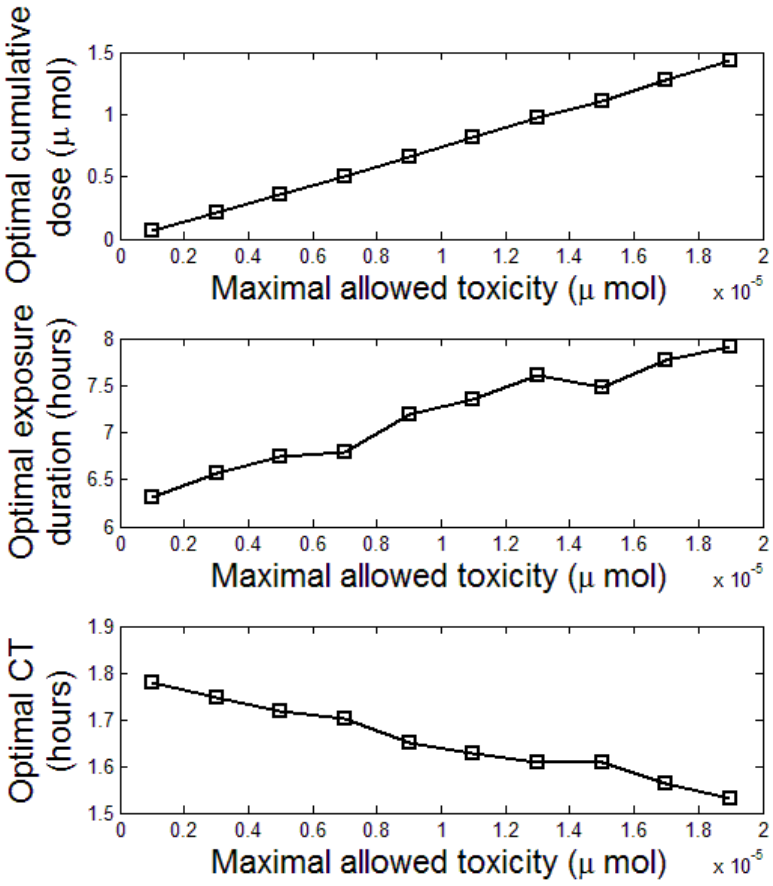
- Trivial exposure scheme of short duration (no toxicity but no efficacy either)

- Advantage of choosing the right circadian time increases with scheme efficacy (difference between best and worst circadian times of exposure for durations between 4 and 6 hours)

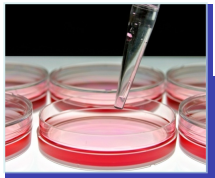


Optimal control for Irinotecan exposure: *Maximizing efficacy under constraint of toxicity*





- Optimal dose increases linearly with maximal allowed toxicity
- Optimal CT between CT 1.5 and 1.8, optimal duration 6 to 8 hours



Conclusion of this experimental identification work

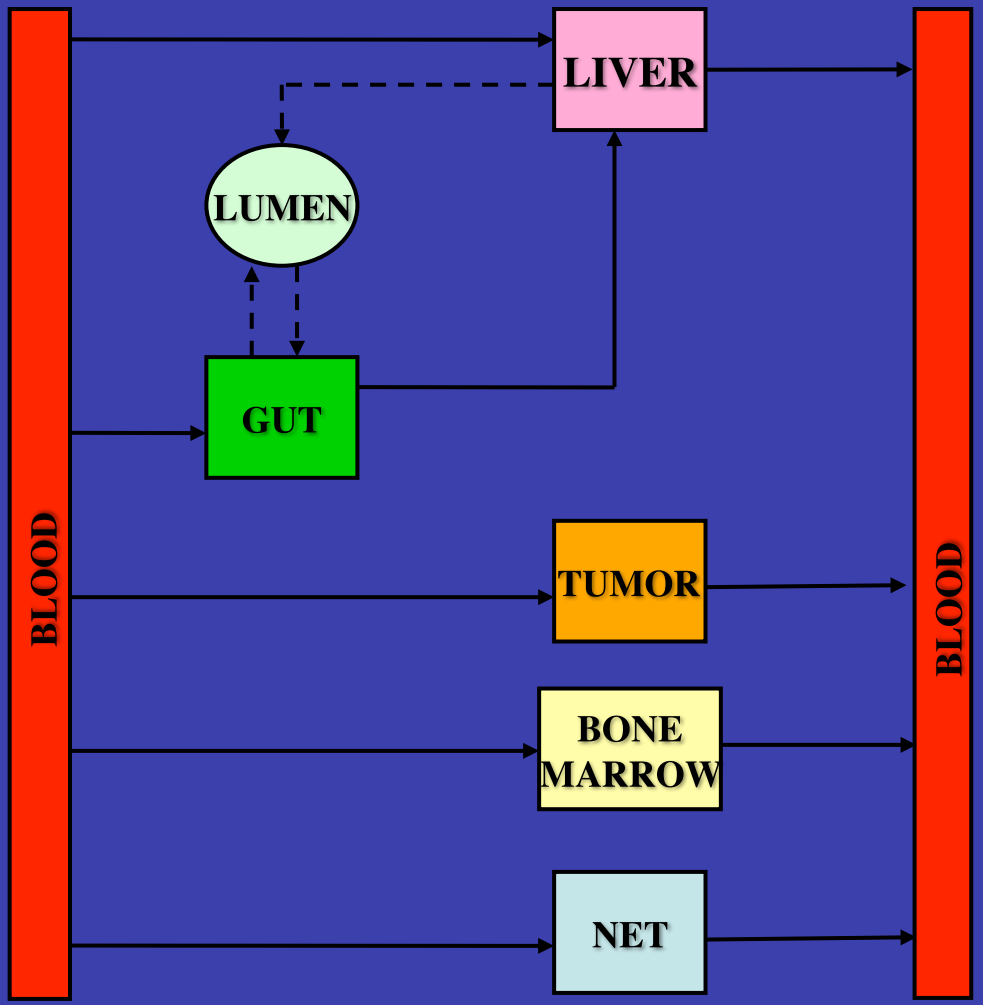
- A mathematical model for CPT11 molecular PK-PD and its control by the circadian clock has been designed and fitted to experimental data on Caco2 cells

- Optimal control stategy for a fixed cumulative dose: optimal exposure starting around CT1.6 , during 6 to 8 hours, depending on allowed toxicity

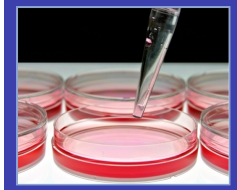
- Future work:
  - CES, UGT1A1 and ABC transporters circadian activities (work in progress)
  - Update optimal exposure schemes and validate them experimentally

Minimal whole body mathematical model in mice

- A whole body physiologically based mathematical model for mice, supplemented with basic cell cycle model
- Each organ contains the tissue level mathematical model built from the cell culture study



Summary and future work



- Optimisation of exposure on cell cultures
  - Built the mathematical model at tissue level
  - Detailed parameter estimation
  - Validation of the mathematical model and of theoretically optimal exposure scheme

- Optimisation of administration in mice
  - Built a whole-body PK-PD model for mice
  - Parameter estimation (starting from cell culture values): one set of parameter for each one of 3 different mouse strains
  - Validation of mathematical model and of theoretically optimal administration schemes

- Future: optimisation of administration to patients
  - Adaptation of the whole-body model.
  - Parameter estimation : one set of parameter for each class of patients (e.g. men, women) or patient
  - Validation of theoretically optimal administration scheme

Toward whole body physiologically based PK-PD ("WBPBPKPD") modelling and model validation

Controlling cell proliferation for medicine *in the clinic* is a multiscale problem, since drugs act at the single cell and cell population levels, but their clinical effects are measured at a single patient (=whole organism) and patient population levels

1. Drug detoxification enzymes, active efflux, etc.: molecular PK-PD ODEs, with validation by biochemistry data collection and in vitro experiments

2. Drug effects on cells and cell populations: averaged molecular effects on cell proliferation PDE models, with validation by measures of growth in cell cultures

3. Drug effects at the organism level: *WBPBPKPD* modelling: compartmental ODEs, with validation by tissue measurements: animal experiments, clinical trials

4. Interindividual variations (genetic polymorphism): discriminant and cluster analyses on populations of patients (populational PK-PD to individualise therapies)

5. Optimisation of treatments: optimisation methods, with validation by clinical trials