Proliferation in Cell Population Models with Age Structure

Frédérique Billy[*], Jean Clairambault[*], Olivier Fercoq[†], Stéphane Gaubert[†], Thomas Lepoutre[**] and Thomas Ouillon[‡]

[*]*INRIA Paris-Rocquencourt & LJLL, Université Pierre-et-Marie-Curie, 4 Pl. Jussieu, F75005 Paris*
[†]*INRIA Saclay-Île-de-France & CMAP, École Polytechnique, 91128 Palaiseau Cedex, France*
[**]*INRIA Rhône-Alpes & Université Lyon 1, 43 Bd. du 11 novembre, 69622 Villeurbanne Cedex, France*
[‡]*ENSTA ParisTech, 32 Boulevard Victor, 75739 Paris Cedex 15, France*

Abstract. We study proliferation in tissues from the point of view of physiologically structured partial differential models, focusing on age synchronisation in the cell division cycle in cell populations and its control at phase transition checkpoints.

We show how a recent fluorescence-based technique (FUCCI) performed at the single cell level in proliferating cell populations allows identifying model parameters and how it may be applied to investigate healthy and cancer cell populations.

We show how this modelling approach allows designing original optimisation methods for cancer chronotherapeutics, by controlling eigenvalues of differential operators underlying proliferation dynamics, in tumour and in healthy cell populations.

Keywords: Cell population dynamics, Partial Differential Equations, Age-structured Models, Cell division cycle, Growth exponent, Cancer, Circadian rhythms, Therapeutic optimisation
PACS: 87.17.Aa, 87.17.Ee

AGE-STRUCTURED MODELS FOR TISSUE PROLIFERATION AND ITS CONTROL

Tissue proliferation in living organisms always relies on the cell division cycle: one cell becomes two after a sequence of molecular events that are physiologically controlled at each step of the cycle at so-called checkpoints[1]. This process occurs in all renewing tissues, healthy or tumour, but in tumour tissues part of these control mechanisms are inefficient, resulting in uncontrolled tissue growth which may be given as a definition of cancer.

Circadian clocks are known to physiologically control cell proliferation and their disruption has been reported to be a possible cause of lack of control on tissue proliferation in cancer [2].

The representation of the dynamics of the division cycle in proliferating cell proliferations by physiologically structured partial differential equations (PDEs), which dates back to McKendrick [3], is a natural frame to model proliferation in cell populations, healthy or tumour. Furthermore, the inclusion in such models of targets for its physiological and pharmacological control allows to develop mathematical methods of their analysis and therapeutic control [4, 5], in particular for cancer chronotherapeutics when the drug control is made 24h-periodic to take advantage of favorable times, due to circadian clocks.

Physiologically structured cell population dynamics models have been extensively studied in the last 20 years, see e.g. [6, 7]. We consider here typically age-structured cell cycle models. If we consider that the cell division cycle is divided into I phases (classically 4: G_1, S, G_2 and M), we follow the evolution of the densities $n_i(t,x)$ of cells having age x at time t in phase i. Equations read :

$$\begin{cases} \frac{\partial n_i(t,x)}{\partial t} + \frac{\partial n_i(t,x)}{\partial x} + d_i(t,x)n_i(t,x) + K_{i\to i+1}(t,x)n_i(t,x) = 0, \\ n_{i+1}(t,0) = \int_0^\infty K_{i\to i+1}(t,x)n_i(t,x)dx, \\ n_1(t,0) = 2\int_0^\infty K_{I\to 1}(t,x)n_I(t,x)dx. \end{cases} \quad (1)$$

together with an initial condition $(n_i(t=0,.))_{1\le i\le I}$. This model was first introduced in [8]. The particular case $I = 1$ has received particular attention from the authors [9, 10]. In this model, in each phase, cells are ageing with constant speed 1 (transport term), they may die (with rate d_i) or go to next phase (with rate $K_{i\to i+1}$) in which they start with age 0. We write it in its highest generality. If we want to represent the known effect of circadian rhythms on phase transitions [2], we will consider time-periodic coefficients d_i and $K_{i\to i+1}$, the period being in this case 24h.

BASIC FACTS ABOUT AGE-STRUCTURED LINEAR MODELS

One of the most important facts about linear models is the trend of their solutions to exponential growth. Solutions to (1) satisfy (if the coefficients are time-periodic, or stationary) $n_i(t,x) \sim C^0 N_i(t,x)e^{\lambda t}$ [11], where N_i are defined by

$$
\begin{cases}
\frac{\partial N_i(t,x)}{\partial t} + \frac{\partial N_i(t,x)}{\partial x} + \lambda N_i(t,x) + d_i(t,x)N_i(t,x) + K_{i \to i+1}(t,x)N_i(t,x) = 0, \\
N_{i+1}(t,0) = \int_0^\infty K_{i \to i+1}(t,x)N_i(t,x)dx, \\
N_1(t,0) = 2\int_0^\infty K_{I \to 1}(t,x)N_I(t,x)dx, \\
N_i > 0, \quad N_i(t+T,.) = N_i(t,.), \quad \sum_i \int_0^T \int_0^\infty N_i(t,x)dxdt = 1.
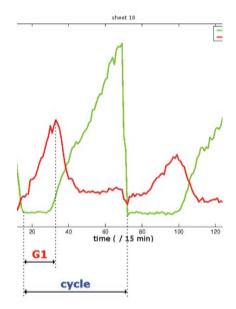\end{cases}
\tag{2}
$$

with $T-$periodic coefficients. The study of the growth exponent, first eigenvalue of the system, is therefore crucial.

In this part, we focus on the case of stationary phase transition coefficients ($K_{i \to i+1}(t,x) = K_{i \to i+1}(x)$) and we do not consider death rates. As shown in [8], the first eigenvalue λ is then solution of the following equation, which in population dynamics is referred to, in the 1-phase case ($I = 1$) with no death term, as Lotka's equation:

$$
\frac{1}{2} = \prod_{i=1}^{I} \int_0^{+\infty} K_{i \to i+1}(x)e^{-\int_0^x K_{i \to i+1}(\xi)d\xi}e^{-\lambda x}\, dx
\tag{3}
$$

We recall that, integrating equation (1) along its characteristics, we can in the stationary case with no death rate derive the formula: $n_i(t+x,x) = n_i(t,0)e^{-\int_0^x K_{i \to i+1}(\xi)d\xi}$. This can be interpreted in the following way: *the probability that a cell which entered phase i at time t stay for at least an age duration x in phase i is given by* $P(\tau_i \geq x) = e^{-\int_0^x K_{i \to i+1}(\xi)d\xi}$. The time τ_i spent in phase i is thus a random variable on $[0,+\infty[$, with probability density function f_i given by: $dP_{\tau_i}(x) = f_i(x)dx = K_{i \to i+1}(x).e^{-\int_0^x K_{i \to i+1}(\xi)d\xi}dx$, whence, equivalently, $K_{i \to i+1}(x) = \dfrac{f_i(x)}{1 - \int_0^x f_i(\xi)d\xi}$.

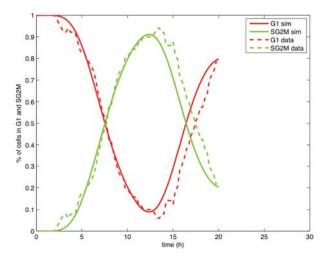# FUCCI (CELL CYCLE) REPORTERS TO IDENTIFY MODEL PARAMETERS

FIGURE 1. Graphic method used to determine the durations of the whole cell cycle and of G_1 phase. The duration of phase $S/G_2/M$ was deduced by subtracting the duration of phase G_1 from the duration of the cell cycle.

FUCCI is a recent cell imaging technique that allows tracking progression within the cell cycle of an individual cell [12]. We used FUCCI data, that consisted in time series of the intensity of red and green fluorescence emitted by

individual NIH 3T3 cells (mouse embryonic fibroblasts) within an *in vitro* homogeneous population proliferating in a liquid medium. This allowed us to measure the time an individual cell spent in the G_1 phase and in the phases $S/G_2/M$ of the cell cycle. Our data consisted of cell cycle phase durations from 55 proliferating cells. A graph representing a time series from an individual cell and the method used to record phase durations is presented on Figure 1.
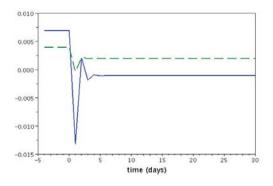
We used these experimental data to identify the parameters of our model by fitting shifted Gamma distributions $f(x) = \rho^{-k}(\Gamma(k))^{-1}(x-a)^{k-1}e^{-\frac{(x-a)}{\rho}}$ on $[a, +\infty[$ to recorded G_1 and $S/G_2/M$ durations, and compared the solutions of the system with cell recordings, that had previously been synchronised by hand. The result is shown on Figure 2.

FIGURE 2. Time evolution of the percentages of cells in the phases G_1 (deep grey) and $S/G_2/M$ (light grey) from biological data (dashed line) and from numerical simulations (solid line). Our model results in a reasonably good fit to biological data.

OPTIMISING EIGENVALUES AS OBJECTIVE AND CONSTRAINT FUNCTIONS

We then used combined time-independent data on phase transition functions, obtained from model identification, with cosine-like functions representing the periodiic control on these transitions by circadian clocks, together with free-running drug infusion regimens that were optimised using a projected gradient method, aiming at decreasing the growth rate in a cancer cell population (objective) while preserving the same in a healthy cell population (constraint).

FIGURE 3. Daily mean growth rates for cancer (solid line) and healthy cells (dashed line) when starting drug infusion at time 0. After a 10-day transient phase, the drug-forced biological system stabilizes towards the expected asymptotic growth rate

Transitions from one phase to the other are described by the transition rates $K_{i \to i+1}(t,x)$. We took them with the form $K_{i \to i+1}(t,x) = \kappa_i(x)\psi_i(t)(1 - g_i(t))$ where $\kappa(x)$ is the transition rate of the cell without circadian control identified in the previous section, $\psi_i(t)$ is the natural circadian control modelled by a cosine and $g_i(t)$ is the effect at the cell level of the drug infusion at time t on the transition rate from phase i to phase $i+1$. No drug corresponds to $g_i(t) = 0$, a transition-blocking infusion corresponds to $g_i(t) = 1$.

The result of an optimised drug infusion regimen $g_i, i = 1,2$ on both growth rates is shown on Figure 3. On this figure, it can be seen that the asymtotic growth rate of cancer cells, initially positive and higher than the one of healthy cells, has been made negative by the periodic treatment exerted on transition rates while the new growth rate of healthy cells, though moderately affected by the treatment, remains positive.

CONCLUSION AND FUTURE PROSPECTS

Taking advantage of quantitative measurements obtained by performing recent image analysis techniques of the cell division cycle in individual cells inside a population of proliferating cells of the same healthy lineage, we have focused in this paper on age synchronisation of cells with respect to cell cycle phases and periodic control on phase transitions.

The proliferation dynamics of this cell population is well approximated by Gamma distributions for cycle phase durations, for which the growth exponent λ, first eigenvalue of the system, can be computed and controlled.

Assuming a multiplicative combination for both temporal controls, physiological (circadian) and pharmacological, on cell cycle phase transitions in a 2-phase McKendrick model of proliferation, we propose a new therapeutic optimisation scheme under a toxicity constraint to control growth exponents in both cancer and healthy cell populations.

By using this combination of physiologically based modelling of proliferation, mathematical analysis methods, cell imaging and statistical parameter identification techniques, and original optimisation algorithms using eigenvalue control of growth processes, we propose a rationale for therapeutic optimisation in oncology at the molecular level in cell populations, healthy and tumour.

By using more FUCCI data on both cancer and healthy cell populations in the future, we should be able to compare synchronisation and control in different settings in these two situations. In this way, using the methodology described in this study, we should be able to examine the part played by circadian clocks in synchronisation of cells in a given population, and to propose new therapeutic tracks in oncology, in particular in cancer chronotherapeutics.

REFERENCES

1. D. Morgan, *The Cell Cycle: Principles of Control*, Primers in Biology series, Oxford University Press, 2006.
2. F. Lévi, A. Okyar, S. Dulong, P. F. Innominato, and J. Clairambault, *Annual Review of Pharmacology and Toxicology* **50**, 377–421 (2010).
3. A. McKendrick, *Proc. Edinburgh Math. Soc.* **54**, 98–130 (1926).
4. J. Clairambault, *IEEE-EMB Magazine* **27**, 20–24 (2008).
5. F. Billy, J. Clairambault, O. Fercoq, S. Gaubert, T. Lepoutre, and T.Ouillon, Synchronisation and control of proliferation in cycling cell population models with age structure (2011), submitted.
6. J. Metz, and O. Diekmann, *The dynamics of physiologically structured populations*, vol. 68 of *Lecture notes in biomathematics*, Springer, New York, 1986.
7. O. Arino, *Acta Biotheor.* **43**, 3–25 (1995).
8. J. Clairambault, B. Laroche, S. Mischler, and B. Perthame, A mathematical model of the cell cycle and its control, Tech. rep., INRIA, Rocquencourt (France) (2003), URL http://hal.inria.fr/view_by_stamp.php?label=INRIA-RRRT&langue=fr&action_todo=view&id=inria-00071690&version=1.
9. J. Clairambault, S. Gaubert, and T. Lepoutre, *Mathematical Modelling of Natural Phenomena* **4**, 183–209 (2009).
10. J. Clairambault, S. Gaubert, and T. Lepoutre, *Mathematical and Computer Modelling* **53**, 1558–1567 (2011).
11. B. Perthame, *Transport Equations in Biology*, Frontiers in Mathematics series, Birkhäuser, Boston, 2007.
12. A. Sakaue-Sawano, K. Ohtawa, H. Hama, M. Kawano, M. Ogawa, and A. Miyawaki, *Chem Biol.* **15**, 1243–48 (2008).