Stochastic modeling and simulation of flow-cytometric labeling experiments reveals distribution of rates of DNA replication

Biao Li[1], Hong Zhao[2], Zbigniew Darzynkiewicz[2], Marek Kimmel[1]

***Short Abstract* — A stochastic model is proposed to simulate flow cytometry data derived from EdU-DNA incorporation time-course experiments. The model aims to reveal different rates of DNA replication at different segments of S-phase. It is designed reproduce the labeling experiments with associated changes in fluorescence intensity of DNA stains. Our findings show that DNA replication in the early segment of S-phase is slower compared to the late S-phase, which is consistent with independently determined DNA synthesis curves.**

***Keywords* — DNA replication rate, flow cytometric analysis, stochastic modeling, simulation**

I. Introduction

DNA replication plays a crucial role in cell cycle kinetics and its understanding is important for cancer research. Modeling as a method of understanding of a biological system has been extensively applied in this field. In the eukaryotic cell, DNA replication is initiated at different replication origins called replicons that are fired at different times during the S-phase [3]. Among a number of stochastic and semi-stochastic approaches that have been used to model cell cycle progression, a few use data derived from flow cytometry labeling experiments [1,2,4]. These models provide estimates of DNA replication rates based on analyzing and evaluating BrdU-DNA flow cytometric histograms in the pulse-labeling cell sample scheme.

However, our data were obtained by using "click chemistry" [5] as a recently introduced approach to assess DNA replication that offers several advantages over the BrdU incorporation methodology [6]. The method is based on the use of EdU with a DNA precursor which allows EdU to penetrate into the specimen more easily than BrdU does, which results in greater accessibility of the EdU to the fluorochrome. Another advantage stems from the fact that no DNA denaturation is required. Detection using this methodology is expected to be more precisely reflecting the actual extent of EdU incorporation (proportional to DNA replication rate) compared to the BrdU incorporation.

We observed, during 30 – 120 min continuous exposure to EdU, the frequency of cells with variable EdU intensities and DNA content. The typical "horseshoe" pattern of the joint distribution of BrdU or EdU and DAPI (this latter proportional to the total DNA content) is observed to have left asymmetry in the measurements at our disposal. In the present study, we approach this observation mathematically, modeling the observed variability in EdU incorporation across the span of the S-phase.

Acknowledgements: This work was funded by NIH grant GM086885.

[1]Departments of Statistics and Bioengineering, Rice University, Houston, TX 77005.

[2]Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, NY 10595.

E-mail: li.biao@rice.edu, kimmel@rice.edu

II. Model and Simulation

The modeling scheme is composed of two steps following a 'top-down' approach. The first is to carry out the simulation of *in vitro* cell growth to mimic the cell culture procedure in order to achieve the 'exponential steady-state' cell population, where proportions of cells in different phases remain the same, and from which cell samples can be drawn. The next step is to simulate DNA labeling. For each dividing cell during S-phase, the time points at which replicons are fired are modeled by a Non-homogeneous Poisson Process (NHPP), assuming shifted gamma distributions for durations of cell cycle phases G_1, S and G_2M. At the present stage, results provide a good fit to the experimental figures suggesting that the NHPP has impact on the labeling intensities of the cell samples. Our findings are consistent with independently determined DNA synthesis rate curves [7].

References

[1] Basse B, Baguley BC, Marshall ES, Wake GC, Wall DJN (2005) Modeling the flow of cytometric data obtained from unperturbed human tumor cell lines: parameter fitting and comparison. *Bull. Math. Biol.*, **67**, 815-830.

[2] Larsson S, Ryden T, Holst U, Oredsson S, Johansson M (2008) Estimating the total rate of DNA replication using branching processes. *Bull. Math. Biol.*, **70**, 2177-2194.

[3] Karp G (2007) Cell and molecular biology: Concepts and experiments. *Wiley*, 5th ed.

[4] Bertuzzi A, Faretta M, Gandolfi A, Sinisgalli C, Starace G, Valoti G, Ubezio P (2002) Kinetic heterogeneity of an experimental tumour revealed by BrdUrd incorporation and mathematical modeling. *Bull. Math. Biol.*, **64**, 355-384.

[5] Darzynkiewicz Z, Zhao H, Halicka HD, Li J (2011) Cytometry of DNA replication and RNA synthesis: Historical perspective and recent advances based on "click chemistry". *Cytometry A*, **79A**, 328-337

[6] Salic A, Mitchison TJ (2008) A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc. Natl. Acad. Sci.*, **105**, 2415-2420

[7] Shaw A, Olivares-Chauvet P, Maya-Mendoza A, Jackson DA (2010) S-phase progression in mammalian cells: modelling the influence of nuclear organization. *Chromosome Res.*, **18**, 163-178