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A quantitative model of the effect of unreplicated DNA on cell cycle progression in frog egg extracts

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ABSTRACT

A critical goal in cell biology is to develop a systems-level perspective of eukaryotic cell cycle controls. Among these controls, a complex signaling network (called 'checkpoints') arrests progression through the cell cycle when there is a threat to genomic integrity such as unreplicated or damaged DNA. Understanding the regulatory principles of cell cycle checkpoints is important because loss of checkpoint regulation may be a requisite step on the roadway to cancer. Mathematical modeling has proved to be a useful guide to cell cycle regulation by revealing the importance of bistability, hysteresis and time lags in governing cell cycle transitions and checkpoint mechanisms. In this report, we propose a mathematical model of the frog egg cell cycle including effects of unreplicated DNA on progression into mitosis. By a stepwise approach utilizing parameter estimation tools, we build a model that is grounded in fundamental behaviors of the cell cycle engine (hysteresis and time lags), includes new elements in the signaling network (Myt1 and Chk1 kinases), and fits a large and diverse body of data from the experimental literature. The model provides a validated framework upon which to build additional aspects of the cell cycle checkpoint signaling network, including those control signals in the mammalian cell cycle that are commonly mutated in cancer.

1. Introduction

The ability of cells to arrest at checkpoints in response to damaged or unreplicated DNA is conserved among eukaryotes (Elledge, 1996), and loss of checkpoints results in genomic instability, which characterizes most human malignancies (Bartek and Lukas, 2003; Hanahan and Weinberg, 2000). Because the core cell cycle machinery as well as the checkpoint signaling networks that affect this machinery are highly conserved, information derived from studying checkpoints in one experimental system, such as the frog egg extract, should provide a framework of knowledge that can be extended to include more complex control circuits in mammalian cells. As details about the molecules that regulate checkpoints and their interactions with one another accumulate, it becomes increasingly difficult to predict how perturbations (such as a deletion or overexpression of a protein) will affect the entire system. Mathematical models provide a system-level view of molecular networks. Experimentally validated models identify critical interactions that control the system and assist in developing the next round of questions to test experimentally.

We and others have used mathematical modeling as a tool to organize a large body of experimental data and discover underlying regulatory principles governing entry into and exit from mitosis in cell-free egg extracts (Novak and Tyson, 1993). The Novák–Tyson model of the cell cycle is based on biochemical kinetics (to generate a set of rate equations for Cdks and their associated proteins) and modern dynamical systems theory (to analyze the solutions of these nonlinear differential equations). The wiring diagram for the model (Fig. 1) summarizes the regulation of cyclin B/Cdk1 by three feedback loops. In egg extracts, newly synthesized cyclin B associates with Cdk1 (present in excess) to form active mitosis-promoting factor (MPF) (Solomon et al., 1990). MPF is rapidly inhibited by phosphorylation on Tyr15 by the kinase, Wee1 (Mueller et al., 1995a). (A second MPF kinase, Myt1, was discovered after the Novák–Tyson model was published (Mueller et al., 1995b). Addition of Myt1 became a critical step in building the model of the DNA replication checkpoint described here.) MPF remains inactive until this phosphate group is removed by the phosphatase, Cdc25 (Gautier et al., 1991; Kumagai and Dunphy, 1991). In turn, active MPF phosphorylates and inhibits Wee1, in a mutually antagonistic feedback loop (Mueller et al., 1995a), and phosphorylates and activates Cdc25, in a positive feedback loop (Izumi and Maller, 1993; Kumagai and Dunphy, 1992). These

Abbreviations: Cdk, cyclin-dependent kinase; MPF, mitosis-promoting factor; NEB, nuclear envelope breakdown; ODE, ordinary differential equation; PET, parameter estimation tools

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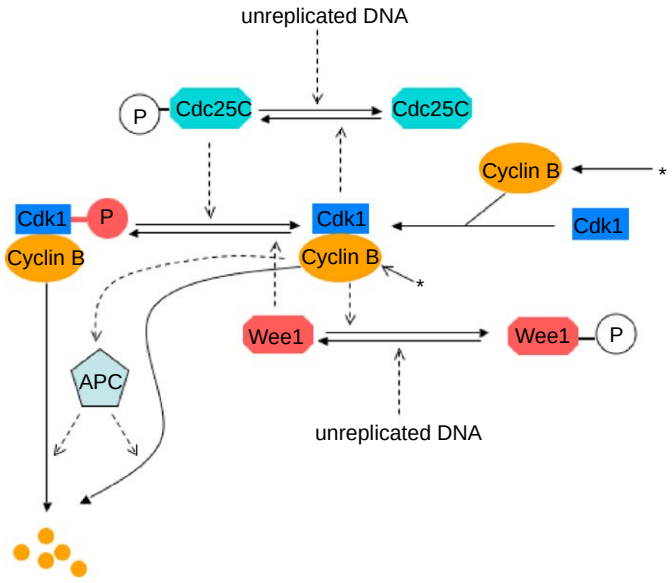

Fig. 1. The Novák–Tyson model of the cell cycle engine in frog egg extracts. Positive feedback between Cdk1 and Cdc25 (Gautier et al., 1991; Izumi and Maller, 1993; Kumagai and Dunphy, 1991; Kumagai and Dunphy, 1992) and double-negative feedback between Cdk1 and Wee1 (Mueller et al., 1995a; Smythe and Newport, 1992) create the abrupt activation of Cdk1 at G2/M. Inactivation of Cdk1 results from negative feedback in which Cdk1 indirectly targets cyclin B for degradation through the Anaphase Promoting Complex (APC) (Lorca et al., 1998). Synthesis of cyclin is represented by an asterisk (*). Computer simulations of the model reproduce oscillations of Cdk1 (MPF) activity as they are observed in cell-free egg extracts (Novak and Tyson, 1993). Novák and Tyson proposed that unreplicated DNA blocks entry into mitosis by activating the phosphatases that oppose MPF's action on Cdc25 and Wee1.

feedback loops are responsible for the abrupt activation of MPF at the G2/M transition. Also important to this control system is a negative feedback loop with time delay in which active MPF indirectly activates the APC to target cyclin B for degradation via the ubiquitin–proteasome pathway (Lorca et al., 1998; Felix et al., 1990).

The Novak–Tyson (1993) model made several predictions about the behavior of the underlying control system regulating entry into mitosis, which we later validated experimentally (Sha et al., 2003). The first prediction we tested was that entry into mitosis (activation of MPF) and exit from mitosis (inactivation of MPF) are regulated by hysteresis. Novák and Tyson predicted that the phosphorylation reactions controlling the activity of MPF can persist in two alternative steady states: an interphase-arrested state with low MPF activity (because of inhibitory phosphorylation of Cdk1), and an M-phase arrested state with high MPF activity (because the inhibitory phosphate group has been removed). The system switches between the two states as the amount of cyclin B in the cell is manipulated (e.g. by altering its rate of synthesis or degradation). Such switch-like behavior is called hysteresis. Hysteresis is created by positive feedback in the underlying control mechanism. Goldbeter's (1991) model, which lacks positive feedback, does not exhibit hysteresis.

In a series of experiments based on Solomon's protocol for MPF activation in frog egg extracts (Solomon et al., 1990), we confirmed that mitosis is governed by hysteresis and bistability. By strictly controlling the amount of cyclin B in the extract (using cycloheximide to block synthesis of endogenous cyclins and adding exogenous non-degradable cyclin at fixed concentrations), we determined that the activation threshold (amount of cyclin B required to activate MPF and drive entry into mitosis) was between 32 and 40 nM, whereas the inactivation threshold (amount of cyclin B required to keep an extract in mitosis) was between 16 and 24 nM (Sha et al., 2003). We showed that, for concentrations of cyclin B just above the activation threshold, the time lag for MPF activation gets very long (a phenomenon called 'critical slowing down'). Thus, by pairing experimental and computational studies, we established a fundamental principle of cell cycle control in cell-free egg extracts. Pomerening et al. (2003) confirmed this principle in an independent study.

When a DNA replication checkpoint is engaged, stalled DNA replication forks trigger a signal transduction network that ultimately causes inactivation of MPF and arrests the cell cycle at the G2/M transition (Nyberg et al., 2002; Qin and Li, 2003; Sagata, 2002). In egg extracts supplemented with DNA, agents that lead to stalled replication forks (e.g. aphidicolin or UV-radiation) activate ATR (Hekmat-Nejad et al., 2000; Lupardus et al., 2002), a kinase that phosphorylates and activates Chk1 (Guo et al., 2000; Kumagai et al., 1998b). In frog eggs, Chk1 promotes cell cycle arrest by phosphorylating Cdc25C and Wee1. Phosphorylation of Cdc25C on S287 by Chk1 causes Cdc25C to bind 14-3-3 protein, sequestering Cdc25C in the cytosol (Kumagai et al., 1998a, b). Phosphorylation of Cdc25C on S287 is mutually exclusive with phosphorylation of Cdc25C on S285 by Cdks (Bulavin et al., 2003a, b). Phosphorylation of Wee1 by Chk1 causes 14-3-3 to bind to Wee1 and stimulates the activity of Wee1 towards Cdks (Lee et al., 2001).

Although little molecular information about the DNA replication checkpoint was available at the time, Novák and Tyson predicted that unreplicated DNA enlarges the hysteresis loop governing mitosis. They placed the effects on the feedback loops involving MPF, Wee1 and Cdc25. Experimentally, we validated this prediction by showing that for a specific concentration of sperm nuclei (1200/μl), an increase in cyclin content from 40 to 100 nM was required to bypass a DNA replication checkpoint (Sha et al., 2003). This discovery of a relatively small quantitative difference (2.5 fold change in cyclin concentration) producing a striking change in physiologic behavior (bypassing cell cycle arrest) provides another example of the novel information gained by pairing experimental studies in egg extracts with mathematical modeling. As we identify points of control in the molecular network that are sensitive to small quantitative changes, we add new layers of understanding about how diseases develop (sometimes by subtle rather than gross changes in gene expression) and might be treated (by adjusting the cell cycle thermostat by degrees, not orders of magnitude). These preliminary studies provided insight into how a checkpoint signaling pathway affects the dynamical behavior of the cell-cycle engine.

To extend our understanding of cell cycle regulation, we address here the effect of unreplicated DNA on the core cell cycle engine, in particular, on the activating phosphatases (Cdc25) and the inhibitory kinases (Wee1 and Myt1) that engage in feedback loops with MPF. In order to preserve the fundamental properties of the control system, the model was constrained to reproduce the phenomena of hysteresis and critical slowing down that we demonstrated experimentally (Sha et al., 2003). Through an iterative process of parameter estimation aided by a newly developed parameter estimation tool (Zwolak et al., 2005a, b), we identified a set of parameter values that brings the model into good qualitative agreement with experiments in which the regulatory phosphatase and kinase (Cdc25 and Wee1) were manipulated in the absence and in the presence of unreplicated DNA. This model serves as a computational tool for investigating the underlying system dynamics of cell cycle checkpoints and predicting the effect of pharmacological and pathological perturbations to the system.