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A predictive mathematical model of the DNA damage G2 checkpoint

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HIGHLIGHTS

- Predictive model of the transition from G2 phase of the cell cycle into mitosis.
- Includes protein phosphorylation sites, locations, concentrations, and activities.
- BioNetGen modeling approach to deal with large number of species and reactions.
- Extensively validated against both published studies and new experimental evidence.
- Likely to provide insight into the design of targeted cancer drugs.

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ABSTRACT

A predictive mathematical model of the transition from the G2 phase in the cell cycle to mitosis (M) was constructed from the known interactions of the proteins that are thought to play significant roles in the G2 to M transition as well as the DNA damage- induced G2 checkpoint. The model simulates the accumulation of active cyclin B1/Cdk1 (MPF) complexes in the nucleus to activate mitosis, the inhibition of this process by DNA damage, and transport of component proteins between cytoplasm and nucleus. Interactions in the model are based on activities of individual phospho-epitopes and binding sites of proteins involved in G2/M. Because tracking phosphoforms leads to combinatorial explosion, we employ a rule-based approach using the BioNetGen software. The model was used to determine the effects of depletion or over-expression of selected proteins involved in the regulation of the G2 to M transition in the presence and absence of DNA damage. Depletion of Plk1 delayed mitotic entry and recovery from the DNA damage-induced G2 arrest and over-expression of MPF attenuated the DNA damage-induced G2 delay. The model recapitulates the G2 delay observed in the biological response to varying levels of a DNA damage signal. The model produced the novel prediction that depletion of pkMyt1 results in an abnormal biological state in which G2 cells with DNA damage accumulate inactive nuclear MPF. Such a detailed model may prove useful for predicting DNA damage G2 checkpoint function in cancer and, therefore, sensitivity to cancer therapy.

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1. Introduction

During the G2 phase of the cell cycle, preparations are made for the transition to mitosis. G2 is the final phase of the cycle where DNA damage can be repaired prior to mitotic cell division (Stark and Taylor, 2006). In response to DNA damage, either endogenous or exogenous, the DNA damage G2 checkpoint pathway is activated. By delaying entry into mitosis and helping to coordinate DNA repair, it plays an important role in protecting cells against genetic alterations that may be involved in tumorigenesis. Attenuation of the DNA damage G2 checkpoint response has been reported in familial cancer syndromes (Hartwell and Kastan, 1994) and cancer (Kaufmann et al., 2008). Several reviews have discussed the G2 to M transition and the G2 checkpoint response to DNA damage (Abraham, 2001; Hutchins and Clarke, 2004; Niida and Nakanishi, 2006; Nilsson and Hoffmann, 2000; van Vugt and Medema, 2005). A consensus diagrammatic description has been developed (Harper and Elledge, 2007), including signaling from the DNA damage sensor checkpoint kinases ATM and ATR to transducer checkpoint kinases Chk1 and Chk2, which act to inhibit cyclin B1/Cdk1 (MPF) kinase activity and exclude MPF from the nucleus, thereby delaying mitotic entry. Since DNA damage G2 checkpoint function may protect against cell killing by radio- and chemotherapies used for cancer, it would be valuable to predict checkpoint function before

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therapy is initiated. Defects in DNA damage checkpoint function have been identified in cancer cell lines (Doherty et al., 2003; Kaufmann et al., 2008). Given the complexity of the interactions among the components of the G2-M transition and the DNA damage G2 checkpoint, it is likely that variation in protein expression levels impacts G2 checkpoint function in cancer cells. Thus a computational model of the G2-M transition and DNA damage G2 checkpoint may be used to predict how variation in protein expression influences checkpoint function (Qu et al., 2003; Tyson et al., 2011). Drawing on the extensive and sometimes contradictory body of experimental evidence available, we have developed a novel computational model of the DNA damage G2 checkpoint. It includes interactions among 16 proteins located in two compartments, their complexes and multiple phosphoforms, described by a set of 138 rules on conditions on complex formation and activities of multiple phosphorylation sites. When fully extended, the reaction network includes 769 species and 2688 reactions. However, the rule-based approach we use allows us to seamlessly manipulate such a large model, testing variable hypothesis, drug effects and parameter variability.

The model is centered on two positive feedback loops: the mutual activation of MPF and Cdc25c and the mutual inhibition of MPF and Wee1. The resulting system exhibits hysteresis, which has also been observed experimentally (Pomerening et al., 2003; Sha et al., 2003). It differs significantly from other G2 checkpoint and cell cycle models (Aguda, 1999; Novak et al., 1998) in including phosphorylation sites as well as nuclear and cytoplasmic compartments. While previous models have included phosphorylation sites (Gerard and Goldbeter, 2009) and compartments (Yang et al., 2006), this model is the first to incorporate both and is substantially more complex than these predecessors. It allows for the determination of whether or not the known reactions are sufficient to account for the experimentally observed behavior of the checkpoint or if additional unknown reactions are required. The compartmentalization of the model allows the investigation of the effects of nuclear import and export on checkpoint function. Furthermore, the model enables the rapid simulation of novel experimental conditions to guide future investigations. Lastly the model allows for the simulation of experiments that, with currently available techniques, are difficult or impossible to carry out at the bench such as experiments to deplete a given protein while altering the activity of another to simulate conditions that may be seen in some cancers or after exposure to a hypothetical agent. This modeling effort provides a platform for conducting in-silico experiments on G2 checkpoint function for both normal human cells as well as cancer cell lines. These types of simulations can be used to help guide experimental investigation as well as verify that our understanding of the protein-protein interactions underlying the G2 to M transition and G2 arrest mechanism is correct. The model not only recapitulates the published behaviors of the G2 to M transition and DNA damage G2 checkpoint (Rieder, 2011; van Vugt and Yaffe, 2010; van Vugt et al., 2010) but also predicts a novel regulation of intracellular trafficking of MPF that requires pkMyt1.

2. Model

2.1. Core model

The modeling of the DNA damage G2 checkpoint was begun by creating a model of the "core" of the checkpoint—the G2 to M transition. The core model is built around two positive feedback loops, a mutual activation loop consisting of MPF (it is assumed for the purpose of this model that CDK1 and cyclin B1 are always found in their bound state) and Cdc25 and a mutual inhibition loop consisting of MPF and Wee1. We assume that substrate and enzyme bind in a reversible reaction to form a complex and that the phosphatase or kinase activity of the enzyme occurs at a rate proportional to the concentration of the complex resulting in the dissociation of the complex.

$$[E]+[S]\leftrightarrow[ES]\rightarrow[E]+[P]$$

The activation of Wee1 and the deactivation of Cdc25 occur spontaneously at a rate proportional to the concentrations of inactive Wee1 and active Cdc25, respectively. As the total amount of Cdc25 is varied, the concentration of active MPF after equilibration switches from a low level to a high level (or vice versa). For appropriate choices of the rate constants this results in a bistable switch—a type of hysteresis where the equilibrium behavior of the system depends on the initial activity level of MPF in addition to the total amounts of the proteins (this bistable behavior is shown in Fig. 1). In a normal G2 to M transition the system will start with low levels of MPF and Cdc25 and a high level of active Wee1. This will result in the immediate deactivation of newly created MPF thus causing the switching to occur at the concentration of Cdc25 determined by the lower curve. The switching behavior demonstrated by this model represents the basic dynamics of the G2-M transition (Novak et al., 2007, 2010).

2.2. Extended model

The core model was extended to include 16 proteins in two compartments, the nucleus and the cytoplasm. The G2 to M

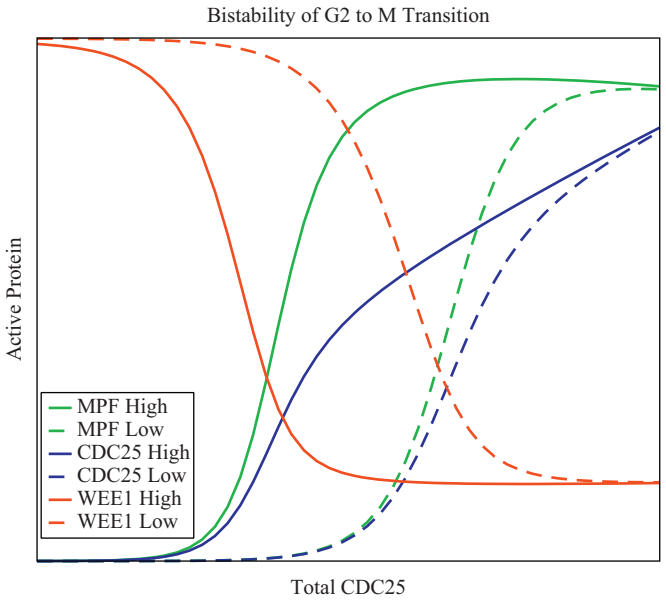

Fig. 1. Bistability of MPF activity in the model during the G2 to M Transition. This figure demonstrates the hysteresis in the core model of the DNA damage G2 checkpoint. The model consists of two positive feedback loops and three proteins: the mutual inhibition of MPF and Wee1 and the mutual activation of Cdc25 and MPF. For a given amount of the signal protein (Cdc25), the equilibrium concentration of the active form of the response protein (MPF) depends on the initial state of the system—in particular, if the MPF was mainly active or mainly inactive at the beginning of the simulation. The solid curves in the figure depict the results of simulations with MPF initially active and the dashed curves represent simulations where MPF was inactive at the outset. The red curves show equilibrium concentrations of active Wee1, the blue curves the amount of active Cdc25, and the green curves show the equilibrium value of active MPF. The total amount of Cdc25 present in the simulation increases along the positive *x*-axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)