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ORIGINAL ARTICLE

Dynamical analysis of the fission yeast cell cycle via Markov chain

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Abstract
The cell cycle is a complex network involved in the regulation of cell growth and proliferation. Intrinsic molecular noise in gene expression in the cell cycle network can generate fluctuations in protein concentration. How the cell cycle network maintains its robust transitions between cell cycle phases in the presence of these fluctuations remains unclear. To understand the complex and robust behavior of the cell cycle system in the presence of intrinsic noise, we developed a Markov model for the fission yeast cell cycle system. We quantified the effect of noise on gene and protein activity and on the probability of transition between different phases of the cell cycle. Our analysis shows how network perturbations decide the fate of the cell. Our model predicts that the cell cycle pathway (subsequent transitions from G1 → S → G2 → M) is the most robust and probable pathway among all possible trajectories in the cell cycle network. We performed a sensitivity analysis to find correlations between protein interaction weights and transition probabilities between cell cycle phases. The sensitivity analysis predicts how network perturbations affect the transition probability between different cell cycle phases and, consequently, affect different cell fates, thus, forming testable in vitro/in vivo hypotheses. Our simulation results agree with published experimental findings and reveal how noise in the cell cycle regulatory network can affect cell cycle progression.

Keywords Transition probability · Cell cycle · Fission yeast · Markov chain · Global sensitivity analysis

Introduction

The biochemical machinery of eukaryotic cells in the cell cycle network allows them to perform cell growth and reproduction. The cell cycle of eukaryotic cells involves consecutive transitions among the cell cycle phases, namely G1, S, G2, and M. Cells with an adequate mass in gap phase G1 can start the cell cycle process by transitioning into the S phase and performing DNA synthesis. Cells in gap phase G2 commit to proceeding to the M phase and reproduce by dividing themselves into daughter cells. The cell cycle network of eukaryotic cells is controlled by complex interactions of key biochemical species including cyclin-dependent kinases and their inhibitors and activators. The concentration of biochemical species that changes during the cell cycle process is responsible for causing the dynamics of subsequent transitions between the cell cycle phases. Although the reaction network controlling the cell cycle has been subject to comprehensive and extensive deterministic models, there are a few reliable stochastic models. Stochastic models can capture the dynamics of biochemical species in the reaction network in the presence of intrinsic noise caused by molecular fluctuations. In this study, we aim to present a stochastic Markov model to investigate the dynamics of noisy cell cycle progression in the fission yeast *Schizosaccharomyces pombe*.

Gene/protein regulatory networks are complex with some components and regulations not yet experimentally

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characterized. For instance, the cell cycle regulatory network of budding yeast is described by approximately 800 interacting genes and proteins, which ensure correct cell division (Spellman et al. 1998). In regulatory networks, a large number of genes and proteins interact with each other and, based on these dynamic interactions, the cell determines its fate and performs its functions (Sundaram and Buechner 2016; Karlebach and Shamir 2008). Regulatory networks are modeled using different mathematical approaches including Boolean (Li et al. 2004), artificial neural networks (Hart et al. 2006; Liu et al. 2017), differential equations, and hybrid approaches (Novak and Tyson 1995; Novak et al. 2001; Tyson et al. 2019). The effects of regulatory uncertainties and molecular noise (Di Talia et al. 2007) in a hypothetical molecular regulatory network can be assessed by using stochastic modeling approaches (Ahmadian et al. 2019, 2020; Tyson et al. 2019) (Braunewell and Bornholdt 2007) (Okabe and Sasai 2007). Although considerable research has been devoted to fission yeast cell cycle regulation (Novak and Tyson 1995) (Novak et al. 2001) (Davidich and Bornholdt 2008) (Castro et al. 2019), the effect of uncertainty on the fission yeast cell cycle regulatory network has not yet been characterized.

We adopted the biological rules of uncertain protein–protein interactions of a first-order Markov model from an existing Boolean model of the fission yeast cell cycle (Davidich and Bornholdt 2008). The Boolean model simulates the transition of a cell between different states without regard to noise or randomness, which is an inherent feature of the cell cycle network. The result of simulation using the Boolean model revealed that the cell cycle system is robust in the absence of noise and the size of the largest attractor of the system (stationary G1) is $\frac{762}{1024}$ [among all of the 1024 initial states that ten binary nodes have in the BN model with 762 initializations, the state of the system reaches a unique stationary G1 state (SG1)]. Our model of the fission yeast cell cycle network describes how uncertain gene/protein interactions robustly control cell transitions between subsequent cell cycle phases.

In general, the dynamic models that are developed to study cell cycle regulation can be divided into several categories (Fuβ et al. 2005), including models that simulate the dynamics of genes/proteins as a discrete/continuous quantity over discrete/continuous time points. Boolean networks (BNs) (Li et al. 2004; Davidich and Bornholdt 2008), probabilistic BNs (Hashimoto et al. 2009), and Markov models (Zhang et al. 2006) are examples of discrete-quantity and discrete-time schemes. Ordinary differential equation models are based on continuous quantities of protein/gene concentration/activity levels during continuous time steps (Boczko et al. 2010; Ferrell Jr et al. 2011; Tyson et al. 2002). Some other methods, based on the Gillespie algorithm (Gillespie 1977) such as stochastic Petri net (Mura and Csikász-Nagy 2008), generate discrete values for genes/proteins during continuous time steps. BNs, as a dynamic discrete system, have been widely used in systems biology for logical analysis of signaling pathways (Fumia and Martins 2013; Lin et al. 2014; Mai and Liu 2009) and gene regulatory networks (Hickman and Hodgman 2009). BNs are made up of interacting nodes in which their states, according to logical rules, are updated synchronously/asynchronously over time. The state of each node is a logical variable, which is either zero (when the gene is inactive or the protein is unphosphorylated) or one (when the gene is active or the protein is phosphorylated) (Sutavani et al. 2018). BNs with simple deterministic rules simulate activatory/inhibitory biochemical interactions between the genes/proteins and predict the dynamics of the state of the genes/proteins, while stochastic models, such as Probabilistic Boolean Networks (PBNs) and Markov models with probabilistic rules, can capture uncertainty and stochasticity (as an inherent feature of biological systems) of gene/protein interactions (Shmulevich et al. 2002; Zhang et al. 2007). If a BN model consists of n binary nodes, it will have 2^n different states where a few of them are attractors (fixed points) within the robust design of the network. It seems that the kinetic parameters related to the structure of gene/protein interactions (such as the weight of the interactions) in a regulatory network affect the robustness of the system and the number and basin size of the attractors (Shafiekhani et al. 2020). Previously, deterministic BN models of the budding yeast (Li et al. 2004) and fission yeast cell cycles (Davidich and Bornholdt 2008) were developed to prove the inherent robustness of the cell cycle network in the absence of molecular noise and variability in protein concentrations. The first-order Markov model of the present study, similar to the BN model of the fission yeast cell cycle, considers the same weight for gene/protein interactions and, using dynamic transition probabilities, predicts the state of the genes/proteins over time. These transition probabilities are derived from the structure of the gene/protein interactions in the fission yeast cell cycle network. In addition to considering the same weight for gene/protein interactions, another simplification that is carried out is to assume the same time scales for the system's biochemical interactions. Ordinary Differential Equation (ODE) models (Novak and Tyson 1995; Novak et al. 2001) and Agent Bases Models (ABMs) (Castro et al. 2019) simulated the cell cycle process by considering the exact time for interactions using many kinetic/dynamic parameters. In ODE-based models, the time evolution of state variables is governed by solving a set of ordinary differential equations while ABMs assign different behaviors and rules for model variables (agents) to capture a system's dynamics through agent–agent interactions. Since, in the present study, we aim to predict the probabilities of transition between different states of the cell cycle, we can ignore the time information of interactions and, by using a Markov model, predict cell state transitions during the process' steps (Zhang et al. 2006).

This paper is organized as follows: In the "Methods" section, we describe how the Markov model was built and simulated based on previously defined rules of protein

interaction. In the "Results" section, we present the simulation results.

Methods

The key constituents of the fission yeast cell cycle network are shown in the Fig. 1 (Davidich and Bornholdt 2008). As shown, ten key proteins (SK, Cdc2/Cdc13, Ste9, Rum1, Slp1, Cdc2/Cdc13*, Wee1/Mik1, Cdc25, and PP) with activatory (green arrows between nodes) and inhibitory (yellow and red dashed arrows) interactions control cell transition between consecutive phases (G1 → S → G2 → M). The interactions between node/protein i and j have specific weights of $w_{ij}, i,j \in [1,2,\ldots,9]$, assuming the indices for i or j are SK: 1, Cdc2/Cdc13: 2, Ste9: 3, Rum1: 4, Slp1: 5, Cdc2/Cdc13*: 6, wee1/Mik1: 7, Cdc25: 8, and PP: 9. In the global sensitivity analysis, we assess the effect of perturbation of these weights on the transition probabilities derived by the Markov model.

In the predictive Markov model, we assume that the current state of the system depends only on the previous state (first-order Markov model), and the probabilities of transition between different states are as follows (Zhang et al. 2006):

$$\Pr\left(S_1(t+1), S_2(t+1), \ldots, S_9(t+1) | S_1(t), S_2(t), \ldots, S_9(t)\right) = \prod_{i=1}^{9} \Pr\left((S_i(t+1) \big| S_1(t), S_2(t), \ldots, S_9(t)\right) \quad (1)$$

$$\Pr\left(S_i(t+1) = k_i | S_1(t), S_2(t), \ldots, S_9(t)\right) = \frac{\exp\left(\beta T_i(2k_i - 1)\right)}{2\cosh\left(\beta T_i\right)} \text{ if} T_i = \sum_{j=1}^{9} w_{ij} S_j(t) \neq 0, k_i = \{0,1\} \quad (2)$$

$$\Pr\left(\mathrm{S}_i(t+1) = k_i | \mathrm{S}_1(t), \mathrm{S}_2(t), \ldots, \mathrm{S}_9(t)\right) = \frac{1}{1+\exp(-\alpha)} \text{if} T_i = \sum_{j=1}^{9} w_{ij} \mathrm{S}_j(t) = 0, k_i = \{0,1\} \quad (3)$$

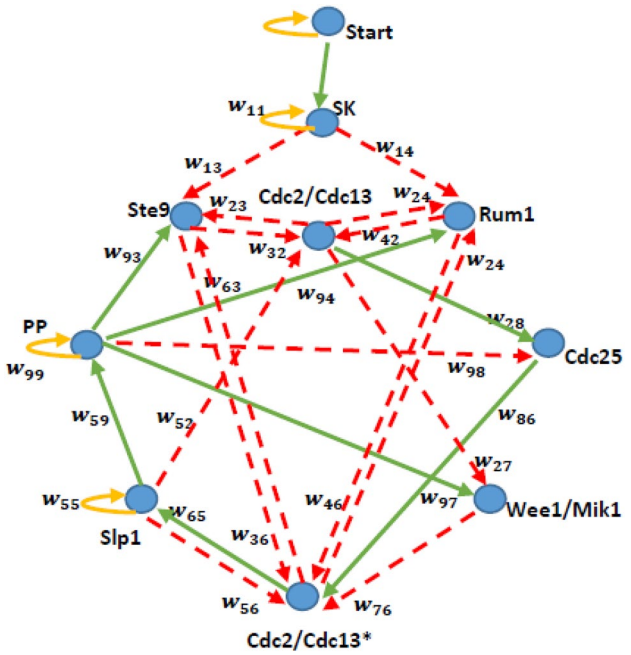

Fig. 1 The cell cycle network of fission yeast (Davidich and Bornholdt 2008). Nodes represent proteins/protein complexes, green/yellow arrows represent activation/self-inhibition effects, and red dotted arrows represent inhibition effects. The values $w_{ij}, i,j \in [1,2,\ldots,9]$ represent the weight of the interaction between protein i and j by assuming the indices i or j are the following: SK: 1, Cdc2/Cdc13: 2, Ste9: 3, Rum1: 4, Slp1: 5, Cdc2/Cdc13*: 6, wee1/Mik1: 7, Cdc25: 8 and PP: 9

We also assume that the cell cycle is active (start = 1) and that the variable states of the fission cell cycle network are SK, Ste9, Rum1, Cdc2/Cdc13, PP, Cdc25, Slp1, Cdc2/Cdc13*, and Wee1/Mik1, which create the dynamics of subsequent cell cycle transitions between different states and the G1, S, G2, and M phases. In Eqs. (1–3), $S_j(t)$ represents the state of protein j at time t, which is zero (inactive/unphosphorylated) or one (active/phosphorylated). For model simplification, the weight of protein–protein interaction, w_{ij}, is either + 1 (activation effect that is depicted by green arrows in Fig. 1) or − 1 (inhibition effect that is depicted by yellow or red dotted arrows in Fig. 1). The state of the proteins at time points $t+1$ is assessed according to their states at time point t and can change or remain unchanged during that time. The parameters β and α in Eq. 2 and 3 are used to uniformly simulate the effect of randomness (noise) and uncertainty for all proteins in cell state transition. If, according to Boolean model rules ($T_i = \sum_{j=1}^{9} w_{ij} S_j(t) \neq 0$), the state of protein S_i at time point $t+1$ must change ($S_i(t+1) \neq S_i(t)$), then the transition probability will be $\frac{\exp\left(\beta T_i(2k_i-1)\right)}{2\cosh(\beta T)}$, $k_i = \{0,1\}$, and the parameter β is used to apply the level of noise in cell state transitions. Here, we note that higher β corresponds to a lower noise level and vice versa. If, according to Boolean model rules, ($T_i = \sum_{j=1}^{9} w_{ij} S_j(t) = 0$), then the state of protein s_i at time point

$t+1$ should not change ($S_i(t+1) = S_i(t)$) and the transition probability will be $1 - \frac{1}{1+\exp(-\alpha)}$ (the parameter α is used to apply the level of noise in cell state transitions). We notice that the lower α corresponds to higher noise and vice versa. In BN, when the state of all proteins does not change, the system tends toward a fixed point or an attractor, while in the Markov model, it is possible to change the state of proteins with a certain probability using the α parameter.

In Fig. 1, nine key proteins/protein complexes responsible for the control and regulation of the fission yeast cell cycle network are depicted (Davidich and Bornholdt 2008). The set of nine binary nodes of BN, namely SK, Ste9, Rum1, Cdc2/Cdc13, PP, Cdc25, Slp1, Cdc2/Cdc13*, and Wee1/Mik1, create $\{0,1\}^9$ state-spaces, including $2^9 = 512$ different states. The evolution of protein/protein complex states in the fission yeast cell cycle regarding noise is modeled via Eqs. (1–3). The Markov model simulates the dynamics of subsequent cell cycle transitions between the 512 different states to mimic the subsequent transitions between the G1, S, G2, and M phases.

As depicted in Fig. 2, we implemented Eqs. for each of the 512 initializations to capture the dynamics of the transition of the proteins/protein complex states during the process. We recorded the state of nine binary proteins/protein complexes for all initializations as a decimal number ($S \in [0,1,2,\ldots,511]$) during the process to create a Markov chain of subsequent transitions of the cell during the cell cycle process. The Markov chain is created using multiple long simulations of the Markov model for all initializations. Therefore, if the chain of subsequent transitions between different states for the ith initialization (ith column of Fig. 2) is $\boldsymbol{X}_i$, $i \in \{0,1,2,\ldots,511\}$, then the Markov chain for the first repetition of model simulation will be $\boldsymbol{X}_{\text{tot}_{\text{rep1}}} = \{\boldsymbol{X}_0, \boldsymbol{X}_1, \ldots, \boldsymbol{X}_{511}\}$. To reduce the effect of aleatoric uncertainty (Shaker and Hüllermeier 2020), we replicated the Markov model simulation several times to create a Markov chain based on multiple long simulations of the Markov

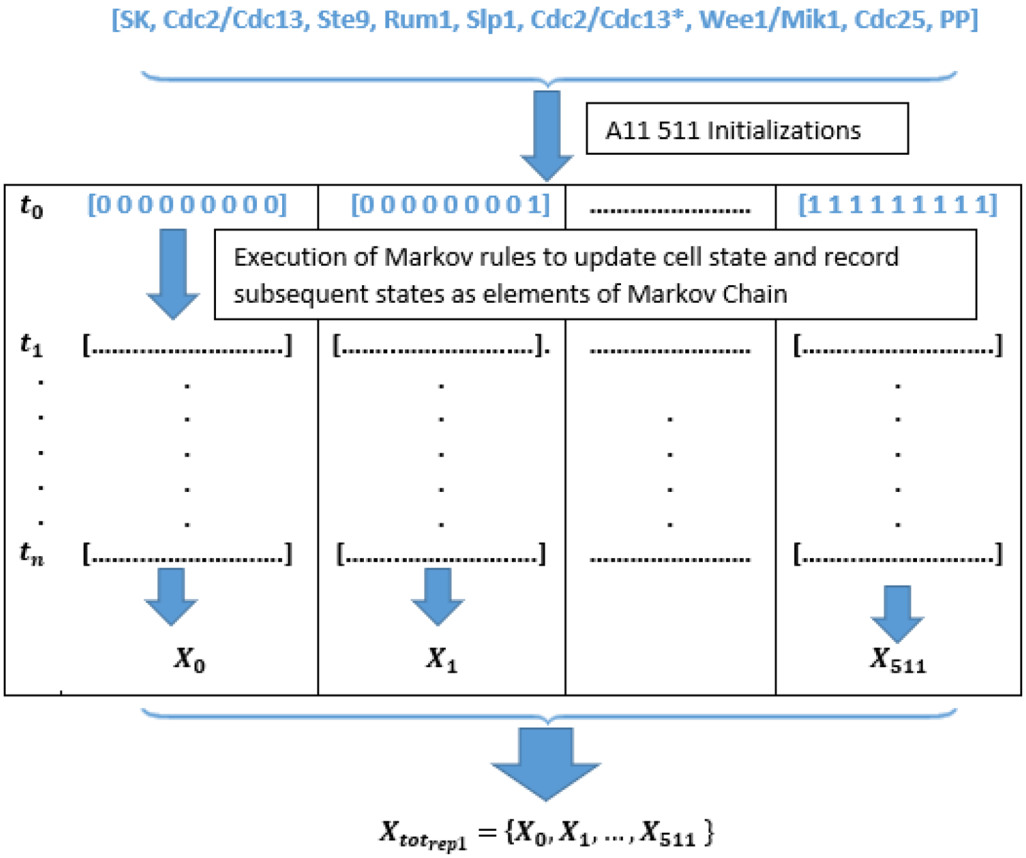

Fig. 2 Schematic diagram of model simulation. One repetition of the Markov model simulation of the fission yeast cell cycle for a11 512 initializations (columns in the figure). For each 512 initial conditions (first row of each column) assigned to the nine proteins (SK, Cdc2 / Cdc13, Ste9, Rum1, Slp1, Cdc2 /Cdc13 *, Wee1 /Mik1, Cdc25, PP) at time t_0 and by applying Markov model rules, the cell dynamics (rows of the table) in the presence of noise over time is predicted. In each time step, the cell state is recorded as a decimal number to create Markov chain $\boldsymbol{X}_{\text{tot\_rep1}}$

model. Then, we used the resulting Markov chain to compute the frequency rate $F_{ij}, i,j \in \{1,2,3,\ldots,512\}$ by counting the number of transitions from state i to state j in one step and computing the one-step transition matrix $P_{ij}, i,j \in \{1,2,3,\ldots,512\}$ as follows: $P_{ij} = \frac{F_{ij}}{\sum_{j=1}^{512} F_{ij}}$, where the element P_{ij} represents the probability of transition from state i to state j in one step.

Global sensitivity analysis (GSA)

We aimed to use global sensitivity analysis (GSA) to investigate model robustness with respect to parameter perturbations and assess the correlation between transition probabilities and model parameters. Therefore, we carried out GSA with respect to the transition probabilities derived by the Markov model, including the weight of protein interactions. Following the GSA method introduced in Marino et al. (2008), we performed Latin hypercube sampling (LHS) and assigned uniform distributions for all 25 parameters (weight of protein interactions), generating 300 samples to compute the partial rank correlation coefficient (PRCC) and their corresponding p values (significance level, p value < 0.05) with respect to the transition probabilities between subsequent cell cycle phases and the steady-state probability of the SG1 state derived by the Markov model. The uniform distribution assigned to each parameter in the LHS method is in the range of $\left[\frac{1}{2}2\right]$. The results of the GSA are presented in the next section.

Results

As shown in Fig. 3, the Markov model predicted the probability of transition from the G1 phase to all states (512 states) for the different process steps and in the presence of a low level of noise ($\alpha = 5$ and $\beta = 4$). As shown in Fig. 3a, the probability of transition from state G1 (356) to all states in one step has the largest probability (0.97) in the G1/S(4) state. Also, as shown in Fig. 3b–h, the probability of transition from G1 (356) to all states in two, three, four, five, six, seven, and eight steps has its largest value in G2(132), G2(130), G2/S (138), G2/S(154), M(19), M(101), and G1(100), respectively. These results are consistent with results from the BN model of the fission yeast cell cycle (Table 1), which, with no regard for noise and uncertainty, simulated the cell cycle's subsequent transitions (Davidich and Bornholdt, 2008). Markov model simulations revealed that the probability of transition between subsequent states

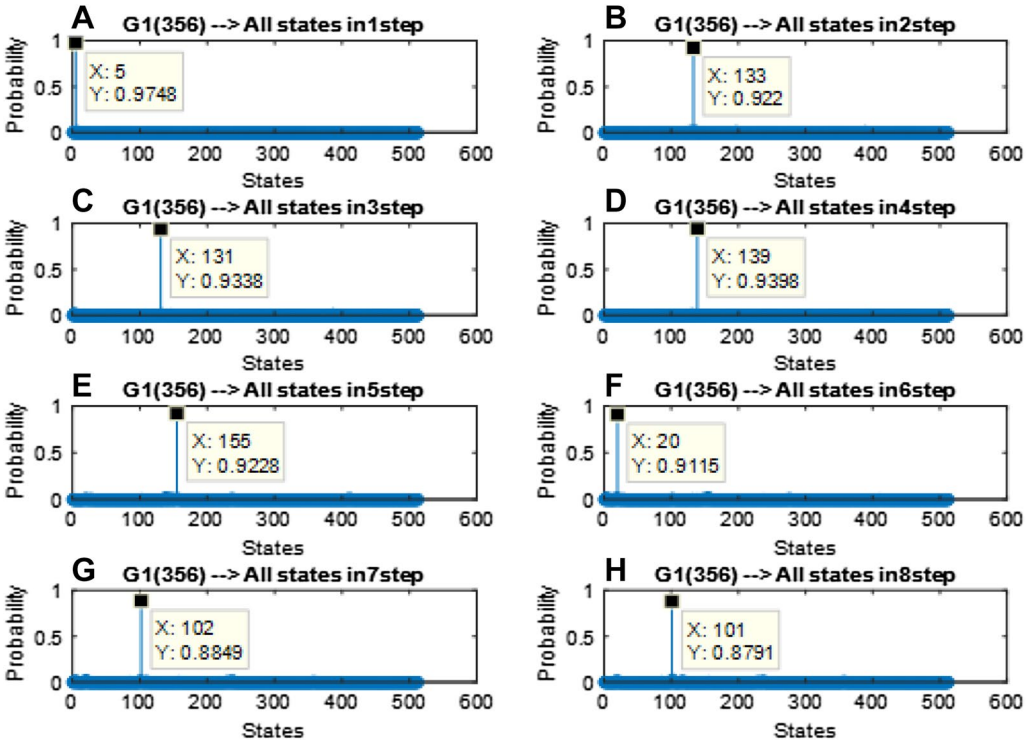

Fig. 3 Transition probabilities from the G1 phase to all states of the fission yeast cell cycle during the process steps with a low level of noise ($\alpha = 5$ and $\beta = 4$). The values of 'X' (horizontal axis) in each subplot reflects the index of each decimal state by subtracting it from one is equivalent to a decimal state of the cell

Table 1 Temporal evolution of protein/protein complex states of the fission yeast cell cycle (Davidich and Bornholdt 2008)

Time step	SK	Cdc2/Cdc13	Ste9	Rum1	Slp1	Cdc2/Cdc13*	Wee1/Mik1	Cdc25	PP	Phase	Decimal State
1	0	0	1	1	0	0	1	0	0	Start	100
2	1	0	1	1	0	0	1	0	0	G1	356
3	0	0	0	0	0	0	1	0	0	G1/S	4
4	0	1	0	0	0	0	1	0	0	G2	132
5	0	1	0	0	0	0	0	1	0	G2	130
6	0	1	0	0	0	1	0	1	0	G2/M	138
7	0	1	0	0	1	1	0	1	0	G2/M	154
8	0	0	0	0	1	0	0	1	1	M	19
9	0	0	1	1	0	0	1	0	1	M	101
10	0	0	1	1	0	0	1	0	0	G1	100

or phases of the cell cycle has its maximum probability among all possible states. For instance, Fig. 3a, shows that the probability of a one-step transition from the G1 state (356) to all states except the G1/S(4) state is about (1−0.97 = 0.03), while the probability of a one-step transition from the G1 state (356) to its next state G1/S(4) is about 0.97.

We investigated the effect of different levels of noise on the dynamics of cell cycle transitions. Therefore, we executed the Markov model for different values of $\alpha \in [3:1:6]$ and $\beta \in [1:1:10]$ to capture the dynamics of the transition probabilities between cell cycle phases/states. As mentioned previously, as the parameters α and β increase, the level of noise decreases. Figures 4, 5, and 6 represent the mean, median, and standard deviation (10 replications of the Markov model assessment) of the transition probabilities (A) Pr(G1(356) → G1/S(4)), (B) Pr(G1/S(4) → G2(132)), (C) Pr(G2(132) → G2(130)), (D) Pr(G2(130) → G2/M(138)), (E) Pr(G2/M(138) → G2/M(154)), (F) Pr(G2/M(154) → M(19)), (G) Pr(M(19) → M(101)), (H) M (101) to SG1 (100), (I) Pr(all states → SG1(100)) in one step. The mean and median of the transition probabilities (for ten replications of the model simulation) have the same trend and values and, as shown in the Figs. 4 and 5, the size of the probabilities increases with increasing α and β values. By increasing these parameters, the uncertainty of the model decreases and the behavior of the stochastic model becomes close to the deterministic BN model. As shown in Fig. 6, the standard deviation of transition probabilities between subsequent cell cycle phases for ten replications of the model simulations are much smaller than the mean of the probabilities.

We also used the Markov model to compute the probability of first passage from state i to state j in $1:t$ steps. If we define T_{ij} as the number of steps taken by the Markov process until first reaching state $X_{T_{ij}} = j$, given that the process initiates with state $X_0 = i$, then the first passage probability from state i to state j in t steps will be as follows:

$$h_{ij}^{t} = P\left(T_{ij} = t\right) = P\left(X_t = j, X_{t-1} \neq j, \ldots, X_1 \neq j | X_0 = i\right)$$
$$h_{ij}^{(1)} = p_{ij},\ h_{ij}^{t} = \sum_{k \in S-\{j\}} p_{ik} \times h_{kj}^{(t-1)} \text{ if } t \geq 2 \tag{4}$$

We used this equation to predict the dynamics of the probabilities of the first transition between subsequent phases of the cell cycle during the process. As depicted in Fig. 7, these probabilities have maximum values in the first step and decrease with increasing steps.

Figure 8a–c shows the mean, standard deviation, and corresponding p values of significant PRCCs for three replications of GSA. The p values shown in Fig. 8c are the maximum p values among the three replications of the GSA analysis. As depicted in Fig. 8a, the probability Pr(G2(132) → G2(130)) among all 25 interactions has only a positive correlation with w_{65} (Cdc2/Cdc13* → Slp1); the probability Pr(G2(130) → G2/S(138)) also has a positive correlation with w_{86} (Cdc25 → Cdc2/Cdc13*). The probability Pr(G2/S(138) → G2/S(154)) has a positive correlation with w_{27} (Cdc2/Cdc13 −| Wee1/Mik1) and w_{86}. The probability Pr(G2/S(154) → M(19)) inversely correlated with w_{55} (Slp1 −| Slp1) and w_{86}, while having a positive correlation with w_{65} (Cdc2/Cdc13* → Slp1) and w_{56} (Slp1 −| Cdc2/Cdc13*). The probability Pr(M(101) → SG1(100)) inversely correlated with w_{99}

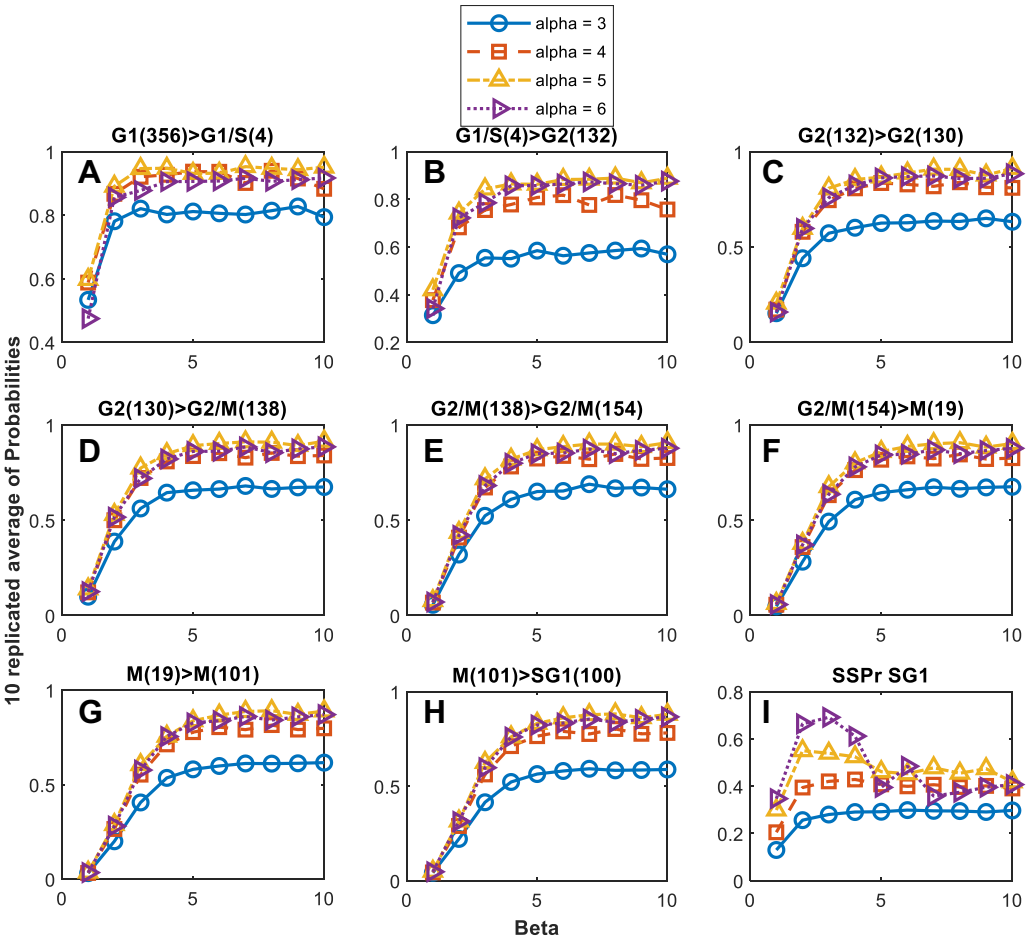

Fig. 4 The average of transition probabilities (10 replications) between two consecutive phases of the fission yeast cell cycle in the presence of different levels of noise ($\alpha \in [3:1:6]$ and $\beta \in [1:1:10]$). **a** Pr(G1(356) → G1/S(4)), **b** Pr(G1/S(4) → G2(132)), **c** Pr (G2(132) → G2(130)), **d** $Pr(G2(130) \rightarrow G2/M(138))$, **e** Pr(G2/M(138) → G2/M(154)), **f** Pr(G2/M(154) → M(19)), **g** Pr(M(19) → M(101)), **h** M (101) to SG1 (100), **i** Pr(all states → SG1(100)) in one step. As the values of α and β increase (decreasing noise), the transition probability between these two consecutive phases increases

(PP − | PP), w_{55}, and w_{86}, while having a positive correlation with w_{59} (Slp1 → PP), w_{65}, and w_{56}. The steady-state probability of the SG1 state Pr(all states → $SG1(100)$) inversely correlated with w_{55}, while having a positive correlation with w_{52} (Slp1 − | Cdc2/Cdc13) and w_{65}

Discussion and conclusion

The primary goal of this study was to quantify the effect of intrinsic noise on transition probability between phases of the cell cycle. We were curious to know how the uncertainty in protein interactions changes the fate of a cell. To this end, we developed a Markov model of the fission yeast cell cycle network to simulate uncertain protein–protein interactions that control cell cycle transitions between subsequent phases. Previously, we showed that the weight of protein–protein interactions in the budding yeast cell cycle network could be optimized to make the cell cycle structure more stable (increase the basin size of stationary G1 as the biggest attractor) and increase the probability of the cell cycle pathway (Pr(G1 → S → G2 → M → SG1)) (Shafiekhani et al. 2020). In this study, we used GSA to assess the relationship between the weights of protein–protein interactions in the fission yeast cell cycle network and the dynamics of transition probabilities derived by the Markov model. Data derived from in silico assessment of the fission yeast cell cycle in GSA analysis match

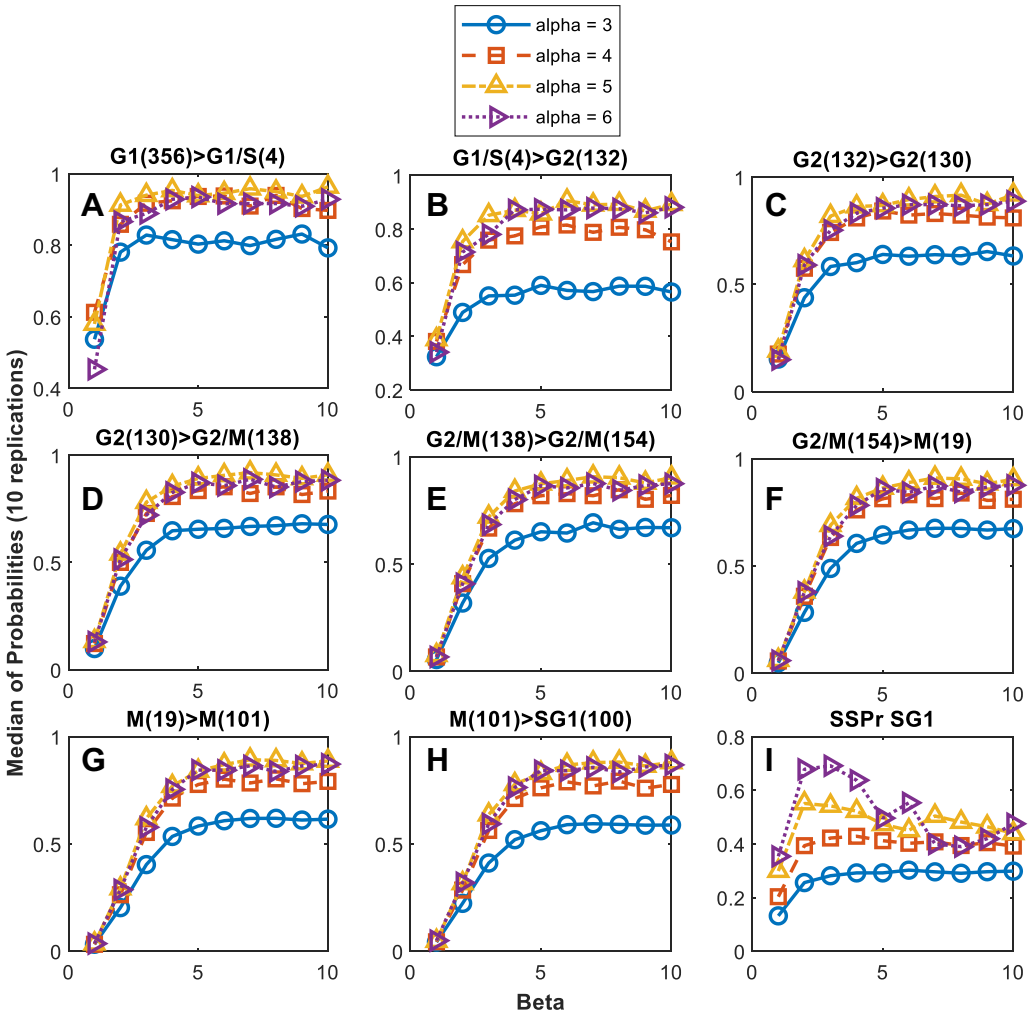

Fig. 5 The median of transition probabilities (10 replications) between two consecutive phases of the fission yeast cell cycle in the presence of different levels of noise ($\alpha \in [3:1:6]$ and $\beta \in [1:1:10]$). **a** Pr(G1(356) → G1/S(4)), **b** Pr(G1/S(4) → G2(132)), **c** Pr (G2(132) → G2(130)), **d** Pr(G2(130) → G2/M(138)), **e** Pr(G2/M(138) → G2/M(154)), **f** Pr(G2/M(154) → M(19)), **g** Pr(M(19) → M(101)), **h** M (101) to SG1 (100), (I) *Pr*(all states → SG1(100)) in one step. As the values of α and β increase (decreasing noise), the transition probability between these two consecutive phases increases

with experimental findings. The results of GSA reveal that progression through the G2/M phase positively correlates with the activity of Cdc25 (w_{86}), which is consistent with the known fact that Cdc25 phosphatase controls G2/M and is a key DNA damage checkpoint regulator. If DNA replication is not completed, cell cycle progression will be halted. Progression through the M phase positively correlates with the activity of Slp1, which is involved in (a) the spindle assembly checkpoint and (b) degradation of Cdc13 by the anaphase-promoting complex and, thus, exits from the M phase and cell division. Notably, progression through the G1 phase does not correlate with any reactions, while, intuitively, we would expect it to have some correlation with the activity of Ste9 and Rum1.

Using GSA, we can test hypotheses about the relationship between the weight of different interactions in the fission yeast cell cycle network and the transition probabilities between subsequent phases of the cell cycle. We aimed to answer the question as to what intervention of the protein interactions (increasing/decreasing the weight of protein interactions) effectively affected the transition probabilities between subsequent phases of the fission yeast cell cycle and the steady-state probability of SG1. For instance, suppose we aimed to identify the best intervention for protein

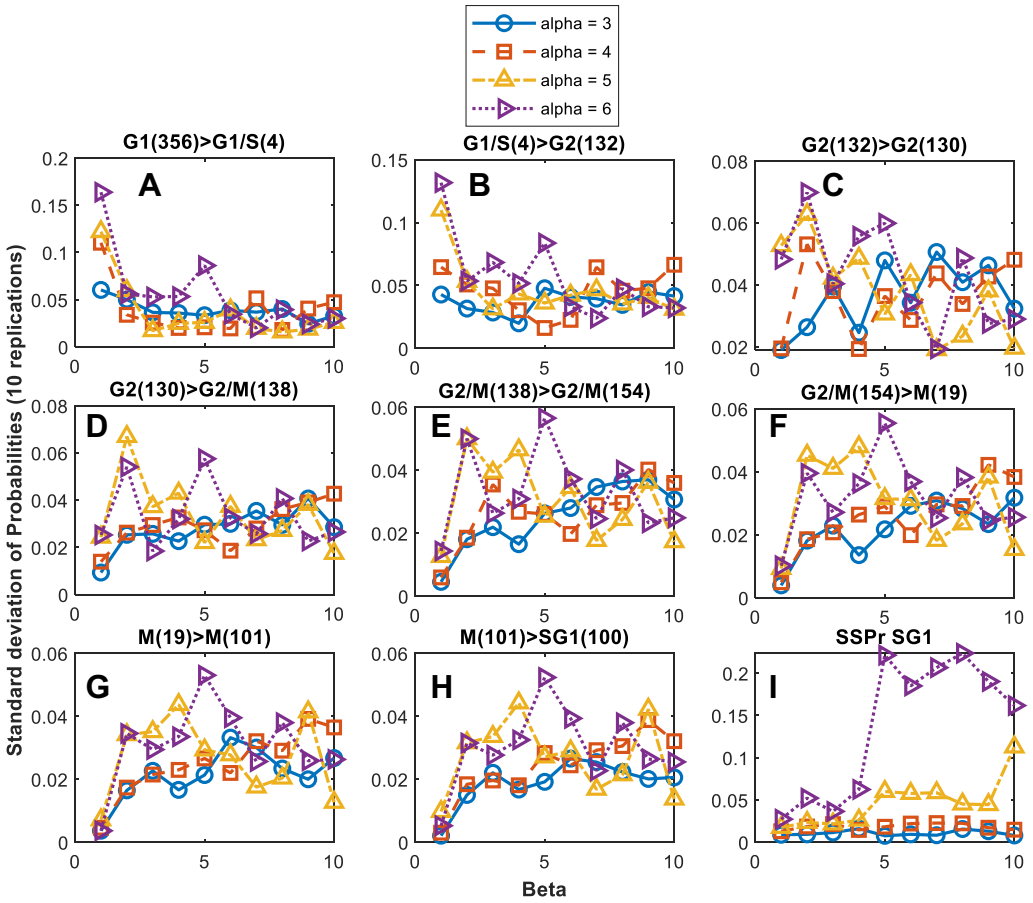

Fig. 6 The standard deviation of transition probabilities (10 replications) between two consecutive phases of the fission yeast cell cycle in the presence of different levels of noise ($\alpha \in [3:1:6]$ and $\beta \in [1:1:10]$). **a** Pr(G1(356) → G1/S(4)), **b** Pr(G1/S(4) → G2(132)), **c** Pr (G2(132) → G2(130)), **d** Pr(G2(130) → G2/M(138)), **e** Pr(G2/M(138) → G2/M(154)), **f** Pr(G2/M(154) → M(19)), **g** Pr(M(19) → M(101)), **h** M (101) to SG1 (100), **i** Pr(all states → SG1(100)) in one step

interactions in the cell cycle process to reduce the probability of cell proliferation. In cancer, malfunctions of the checkpoints of the cell cycle cause some cells with abnormal genetic factors to proliferate in an uncontrollable manner. Thus, identifying a specific intervention for protein interactions to reduce the probability of proliferation can help biologists/immunologists to control tumor growth and eradicate it. We, therefore, designed the GSA. Due to the special importance of changing the cell state from the G2 phase to the M phase, a G2 → M checkpoint prevents the entry of cells with DNA damage or cells in which DNA replication has not been properly performed. We identified interventions of the protein interactions in the fission yeast cell cycle that reduce the probability Pr (G2 → M). Our simulation results showed that one intervention for reducing this probability is to reduce the interaction rate between Cdc25 and the Cdc2/Cdc13* proteins. As shown in Fig. 9, decreasing the weight of the Cdc25–Cdc2/Cdc13* interaction causes G2 arrest in the fission yeast cell cycle. This result is supported by data in Elder et al. (2001) showing that inhibition of phosphorylation of tyrosine 15 on Cdc2 prevents transition from the G2 phase to the M phase and induces G2 arrest in fission yeast. We also predicted that suppression of Slp1 activity induces cell cycle arrest in the M phase. As shown in Fig. 9, the transition probability from G2/M → M is close to zero when Slp1 self-inhibition is high ($w_{55}=2$) or if Slp1 activation ($w_{65}=0.5$) or activity ($w_{56}=0.5$) is low. Slp1 is known to be important for mitotic progression (Matsumoto 1997), which is in agreement with our model prediction.

Many computational models have been developed for dynamical analysis of biochemical networks that control yeast cell cycle progression, including ordinary differential equation models (Novak et al. 2001), Boolean network models (Davidich and Bornholdt 2008; Li et al. 2004),

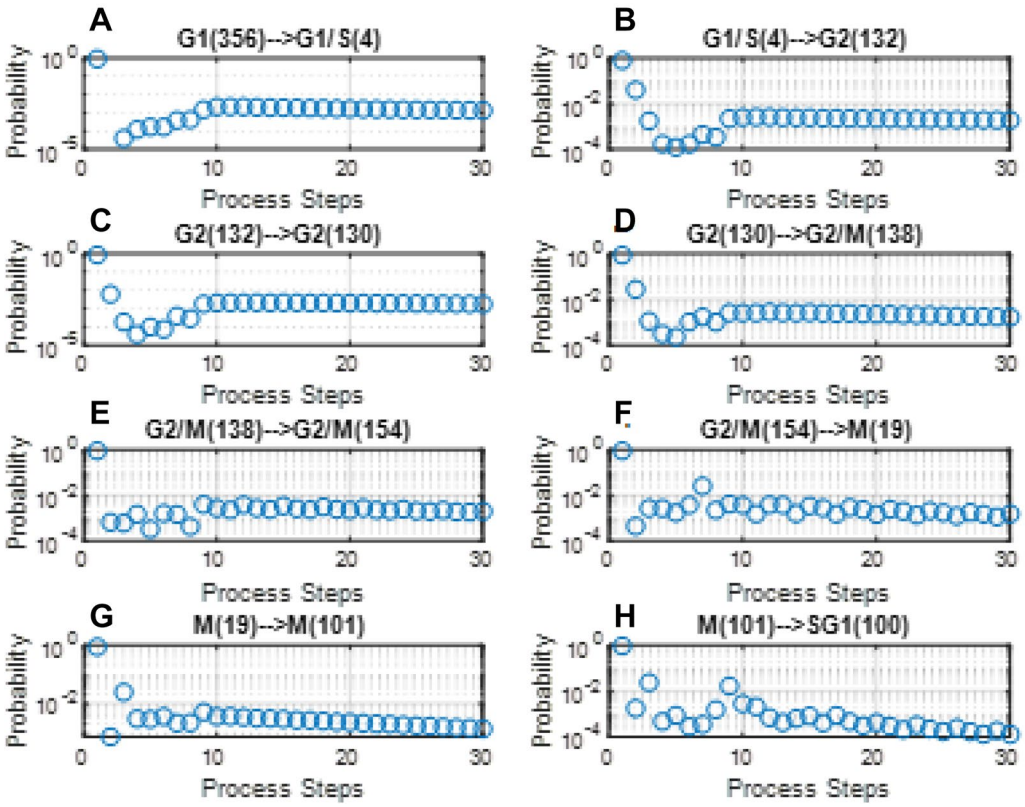

Fig. 7 The probabilities of first passage from one state to another (two subsequent states of the cell cycle) in the presence of a low level of noise ($\alpha = 5, \beta = 4$). **a** Pr(G1(356) → G1/S(4)), **b** Pr(G1/S(4) → G2(132)), **c** Pr (G2(132) → G2(130)), **d** Pr(G2(130) → G2/M(138)), **e** Pr(G2/M(138) → G2/M(154)), **f** Pr(G2/M(154) → M(19)), **g** Pr(M(19) → M(101)), **h** (Pr(M(101) → SG1(100)) in one step. As the values of α and β increase (decreasing noise), the transition probability between these two consecutive phases increases

probabilistic Boolean network models (Hashimoto et al. 2009), Petri net models (Mura and Csikász-Nagy 2008), Markov models (Zhang et al. 2006), and, recently, agent-based models (Castro et al. 2019). The ODE model needs numerous kinetic parameters to simulate the dynamics of gene activity levels/protein concentrations, while the BN model, using simple logical rules (qualitative description of gene/protein interactions) and only using network structure parameters, can simulate the sequence of cell cycle phases, robust behavior of the cell during the cell cycle process, and possible pathways for the cell in the transition from one state to another. The complexity of the BN model is less than that of ODE models, and both models can be used to study the effect of different interventions on protein/gene interactions on cell cycle behavior. On the other hand, these models are deterministic and are not able to simulate the stochastic behaviors of interacting proteins in the cell cycle network. However, probabilistic models, such as the stochastic Petri net, probabilistic BN, Markov model, and agent-based model are capable of simulating stochastic aspects of the cell cycle network.

Castro et al. (2019) presented an agent-based model for simulating the fission yeast cell cycle network that predicted the time duration of different cell cycle phases. The ABM revealed that the time duration of cell cycle phases is similar to that predicted by a previous ODE model (Novak et al. 2001). In another study, Zhang et al. (2006) proposed a stochastic Markov model of the budding yeast cell cycle and examined the robustness of the cell cycle network in the presence of noise. Their simulations revealed that the steady-state probability of transition from all states to the stationary G1 state (the biggest attractor in the budding yeast cell cycle network is SG1) in the presence of noise is significant (0.7); therefore, they proved the robustness of the cell cycle network of budding yeast. Their Markov model focused on the computation of the probability of SG1 and the robustness of the cell cycle of budding yeast. In the present study, we extended their Markov model to simulate the cell

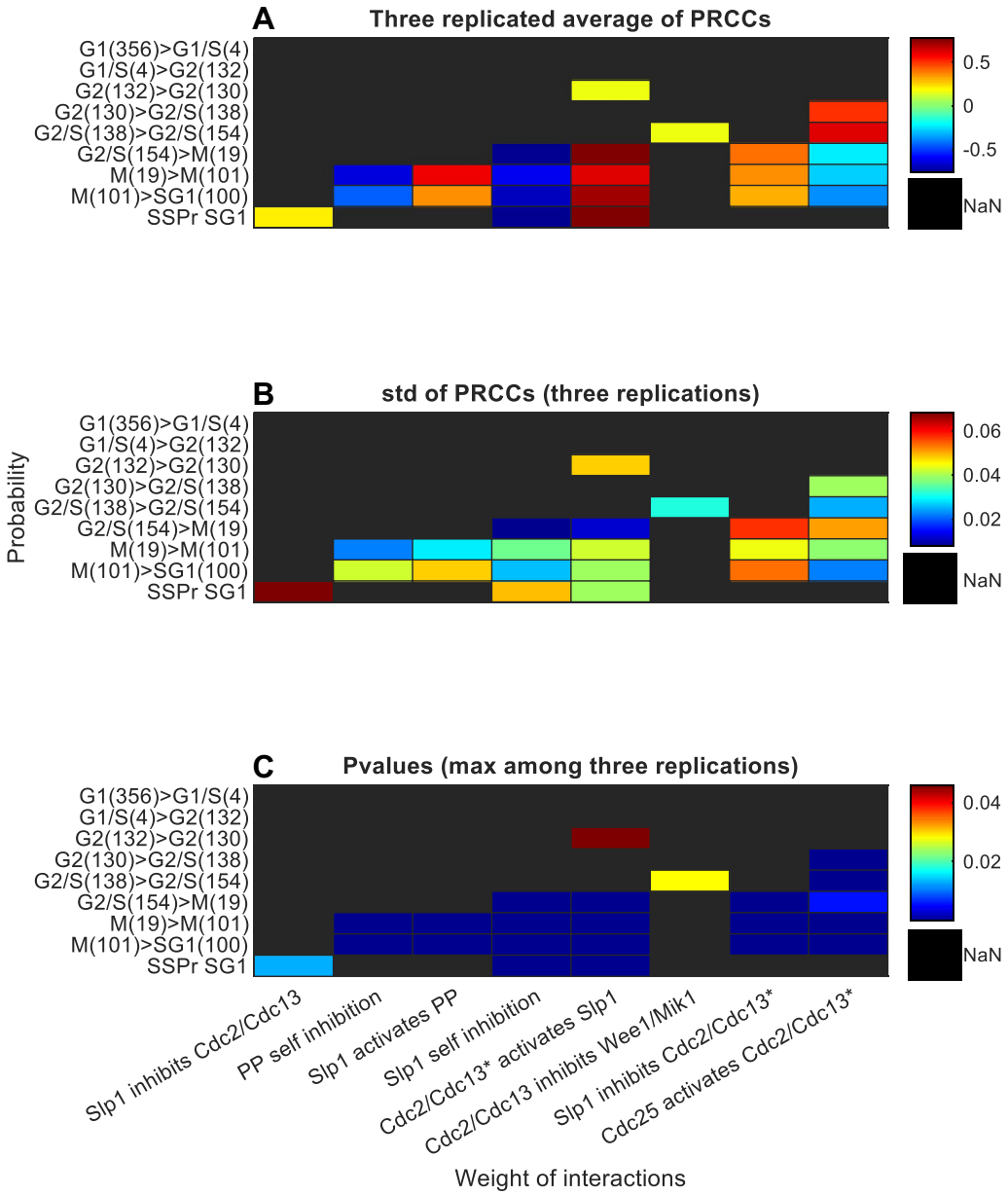

Fig. 8 GSA analysis. **a** Statistically significant PRCC values (p value < 0.05) for transition probabilities between subsequent phases of the fission yeast cell cycle and the steady-state probability of the SG1 state. The mean of the PRCC values for three replications of PRCC analysis (300 runs) is depicted in each pixel. Black pixels ('NaN') show 'not a number' and represent no significant correlation between outcome measures (probabilities, elements in the vertical axis) and the weight of protein interactions of the model (elements in the horizontal axis). **b** The standard deviation of significant PRCC values (p value < 0.05) for three replications of PRCC analyses (300 runs). **c** The maximum of p values for three replications of PRCC analyses (300 runs)

cycle network of fission yeast to capture the dynamics of cell transitions between different cell cycle phases. We used our extended Markov model to investigate the correlation of transition probabilities between subsequent phases of the fission yeast cell cycle network and the weight of protein–protein interactions. Our simulation results are consistent with published experimental findings. Moreover, they provide testable hypotheses so that we can increase our understanding of the cell cycle process by model development and refinement through an iterative in vitro-in vivo/in silico process.

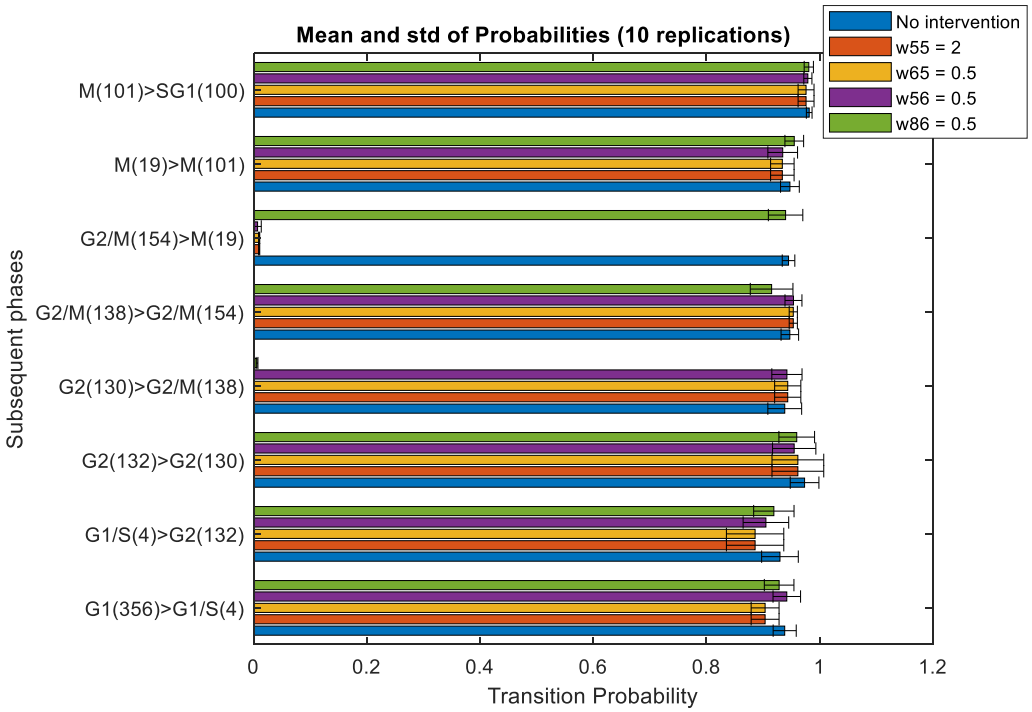

Fig. 9 The effect of change in the degree of protein–protein interactions on transition probabilities between subsequent phases. The effect of the perturbation on the weight of identified protein–protein interactions in GSA on the transition probabilities between subsequent phases. By applying any of the changes: $w_{55}=2$ or $w_{65}=0.5$ or $w_{56}=0.5$ (with respect to no intervention in which all weights are one), the transition probability $\Pr(\mathrm{G2/M}(154) \rightarrow \mathrm{M}(19))$ decreases and becomes close to zero. Also, changing $w_{86}=0.5$, the transition probability $\Pr(\mathrm{G2}(130) \rightarrow \mathrm{G2/M}(138))$ decreases to close to zero

For future work, this model can be extended by adding the messenger RNAs, which are the main source of intrinsic noise in the cell cycle. Also, we can assign fuzzy uncertain numbers instead of crisp values for the weights of protein interactions to capture parametric uncertainty and assess uncertainty in the band of transition probabilities between subsequent cell cycle phases.

Additional Information

MATLAB codes for model simulation will be available by reasonable request to the corresponding author.

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Author contributions SS designed the study, carried out model simulations, analyzed the data, and wrote the paper. PK contributed to the interpretation of the findings and critically revised the paper. MA, AHJ, NG revised the paper. AHJ designed the study and supervised the project.

Conflict of interest The authors declare there is no competing interest.

Ethical approval This study was approved by institutional review board of Tehran University of Medical Sciences.

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