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Quantitative Analysis of a Dynamic Cell Cycle Regulatory Model of *Schizosaccharomyces pombe*

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Abstract

Cell cycle is the central process that regulates growth and division in all eukaryotes. Based on the environmental condition sensed, the cell lies in a resting phase G0 or proceeds through the cyclic cell division process (G1->S->G2->M). These series of events and the irreversible phase transitions are governed mainly by the highly conserved Cyclin dependent kinases (Cdks) and its positive and negative regulators which results in a highly interconnected network. The dynamics of the cell cycle regulation is due to this underlying complex network that governs this process. In *in silico* models it is the parameter set that directly reflects the characteristics of the system. Synthesis rate constants indirectly represent the source of complexity. Therefore, a recently developed model for fission yeast Schizosaccharomyces pombe cell cycle regulation was utilized to investigate the influence of synthesis level regulation on the overall cell cycle period. A systematic local and the global perturbation of sixteen synthesis rate constants of the model were performed to study the synthesis level influence of these regulators on (i) viability, (ii) cell cycle period and (iii) robustness. The results of sensitivity analysis indicates that the cell cycle time is robust to perturbation in the synthesis rate constant of single regulators but fragile to simultaneous perturbation of the multiple regulators. In addition, a perspective on emergence of robustness with respect to multiple layers of complex regulators over a fragile core network is demonstrated based on a systematic regulator deletion and addition analysis. Some of the key predictions that emerge from this study includes, that (i) seven regulatory components Slp1, Cdc2, Cdc13, PP1, APC, and Cdc25 along with Mik1 or Wee1 are sufficient to drive cell cycle regulation. This can be verified by designing appropriate synthetic biology experiments; (ii) either one of the G2 regulatory kinases Wee1 or Mik1 could have emerged through whole chromosome duplication events during evolution which can be tested experimentally to arrive with a conclusive proof.

Keywords: Cell cycle; *S. pombe*; Yeast; Cell Signaling; Evolution; Sensitivity; Robustness

Introduction

The series of process by which a cell replicates its genetic material (S phase) and divides (M Phase) it equally between its daughter is known as cell cycle. This process underlies growth and development in all eukaryotes and is central to their heredity and evolution [1]. Cell cycle regulation is driven primarily by the enzymatic activity of Cyclin dependent kinases (Cdks) and its activation partner cyclin which is universally conserved across eukaryotes [2]. Additional regulations are exerted by several activators and inhibitors through interlinked feedback loops. Information related to the interaction of individual regulators is available through experimental studies and recent times are witnessing the overflow of high throughput experimental data [3-6]. Therefore, systematic modeling approaches that explain the relevance of the underlying biochemical interactions are necessary to better understand the working of the cell cycle network [7].

Theoretical studies have contributed extensively to explore several emerging properties of the cell cycle regulatory networks [8-11]. Robust nature of the biological systems are known, however, the exact underlying mechanisms that contribute towards maintaining robustness is still not well understood and the mathematical foundation is yet to be established [12]. There are very few theoretical studies that investigate the robustness characteristics of the cell cycle through parameter analysis since most of the mathematical studies rely on semi quantitative experimental data for model building [13,14]. Complex systems both engineered and biological are linked with robust yet fragile characteristics that are observed due to modularity. Fragility or failure of a single cell's robust control system leads to fatal disease like cancer [15,16]. It is difficult or almost impossible to experimentally deduce these robust/fragility core of a biological system which could provide insights for drug development studies in complex diseases like cancer [12]. Nevertheless, mathematical models of complex biological processes can be utilized to understand these crucial properties of biological systems.

The present study utilizes a fission yeast *S. pombe* cell cycle regulatory model developed by our group which employed synthesis level regulation for all the regulators [17]. This model demonstrated the wild type dynamics of fission yeast *S. pombe* cell cycle regulators and through simulations predicted the underlying regulatory dynamics of various single, double, temperature sensitive, over-expression and structural mutants. Through structural perturbation studies this model explored the crucial role of multiple phosphatases in imparting specific phenotypic characterizes during cell cycle progression and discussed the ambiguities in the identity and roles of the different phosphatases. In this study through sensitivity analysis the regulatory role of individual regulators and their contribution towards maintaining robust control of fission yeast *S. pombe* cell cycle progression is examined. Oscillatory nature of this dynamic interaction network is known and this study considers it as a measure of viability. We also attempt to integrate this

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information with our systematic multiple deletion analysis that lead to the identification of the core regulators that are essential for maintaining the oscillatory dynamics and thus viability.

Molecular Interaction Map and Model Dynamics

The complex molecular interaction map considered in this study (Figure 1) was utilized in our previous effort to model the fission yeast *S. pombe* cell cycle regulation. A detailed description of the regulatory mechanisms can be found in the same [17]. Nevertheless, a short description and the model dynamics are discussed here. In fission yeast, all regulatory events of the cell cycle are catalyzed by a single Cdk known as Cdc2, which belongs to the serine threonine kinase group of proteins. The Cdk activation in the different phases of the cell cycle happens through phase specific binding of its cyclin partner, which is mediated by cyclin activating kinases (CAK) that phosphorylate Thr 167 of Cdk upon cyclin binding. Three cyclins namely, the G1 specific Puc1, and the S specific Cig2 and mitotic Cdc13 together with Cdc2 precisely regulates different the cell cycle regulation. Rum1, a stoichiometric inhibitor and active Ste9 (APCp-Ste9) are the two negative regulators of G1 phase, that down regulate the M phase specific Cdc2-Cdc13 complex which is also known as the Mitosis Promoting Factor (MPF). While Rum1 forms a complex with active Cdc2-Cdc13 for its proteolysis of the latter, APCp-Ste9 ubiquitinates Cdc13, Cdc2-Cdc13 and marks it for destruction until the cell grows and reaches a critical size. Eventually, the buildup of Cdc2-Puc1 and Cdc2-Cig2 inhibits APCp-Ste9, characterizing the end of G1 phase and the accumulated Cdc2-Cdc13 inactivates Rum1. The level of Cdc2-Cig2 (or SPF) at this state initiates DNA replication. MPF activity and level in S phase and in the subsequent G2 phase are regulated by two negative regulatory kinases namely Mik1 and Wee1, which inactivates it into an inactive preMPF. Increase in the MPF activity at the end of S phase is sufficient to inhibit DNA by suppressing origin re-firing. During the extended G2 phase the gradual increase in the MPF activity positively regulates itself by promoting the activation of a phosphatase Cdc25 and further, negatively regulates the inhibitory kinase Wee1 and Mik1. During the G1, S and until mid G2 phase, Cdc25 is kept inactive by PP1 (just a nomenclature adopted in this study not to be confused with type 1 phosphatase). At the end of G2, active Cdc25 dephosphorylates the accumulated preMPF into active MPF whose level, therefore, rises sharply, marking the transition into the M phase. In the M phase, MPF activates its negative regulator Anaphase Promoting Complex (APC). The hyper-phosphorylated APC (APCp) forms an active complex APCp-Slp1 that marks all forms of cyclins for proteolysis including MPF, thus marking the end of M phase. APCp-Slp1 also promotes activation of Separase, a protease that degrades cohesin rings attached to the sister chromatids by degrading its inhibitor SecurinP.

Upon activation, Separase activates a phosphatase, PP2, which in turn activates Ste9. Ste9 replaces Slp1 in APCp and forms the complex APCp-Ste9. This marks the transition from M phase at the end of cell division into the G1 phase of the subsequent cell cycle. In the absence of MPF mediated inhibition at the end of M phase, PP1 gets activated by auto-dephosphorylation, which in turn activates all negative regulators and inactivates the positive regulator Cdc25. Thus the interwoven complex network described above (Figure 1) governs the dynamic fission yeast *S. pombe* cell cycle regulation. Numerical simulation of the wild type model shows the oscillatory dynamics of various regulatory proteins with a cell cycle period of 150 minutes (Figure 2).

Results

The cell cycle regulatory dynamics observed for the model is manifestation of the molecular interaction networks. It is the kinetic parameters which influences the overall state of the network through individual regulators. How a system gets affected to the changes in its

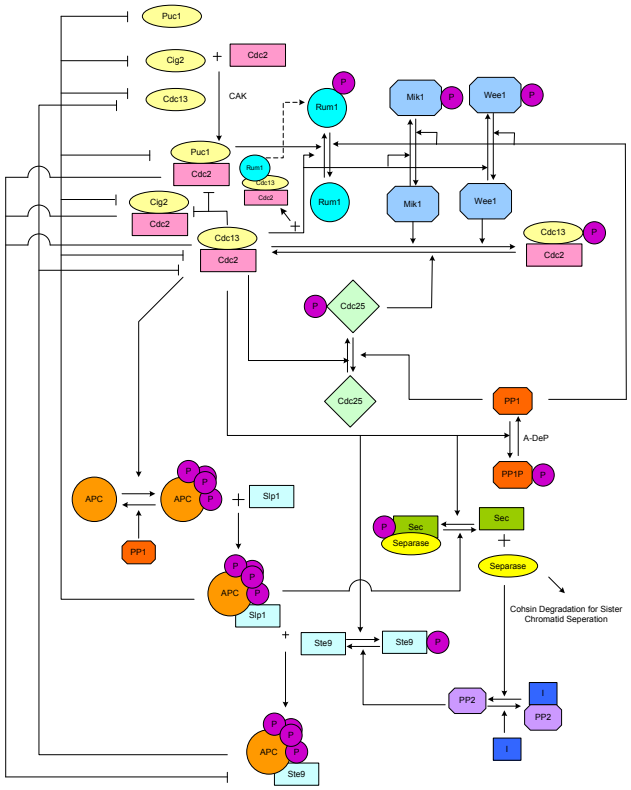

Figure 1: Molecular interaction map of fission yeast *Schizosaccharomyces pombe* that regulates cell division cycle. Note that the fission yeast Cyclin dependent kinases – cylin complex, Cdc2-Cdc13 regulates most of the regulators of this network. The phosphate group '(P)' indicates the phophorylated form of the regulators.

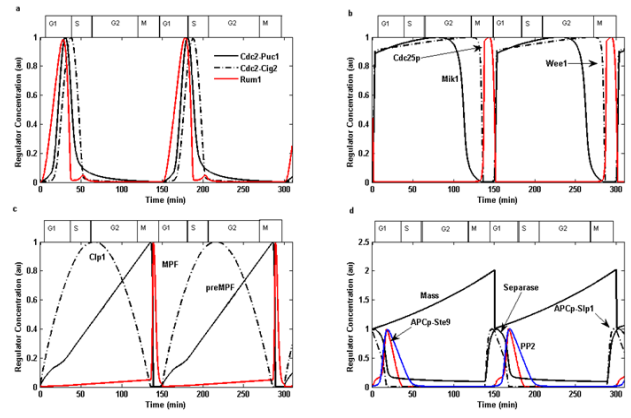

Figure 2: Simulation of wild type fission yeast *S. pombe* cell cycle regulation. The dynamics profiles of sequentially activated and inactivated regulators are seen over a period of two cycles and the cell cycle time is about 150 minutes. All the regulatory protein concentrations are normalized with their respective maximum concentrations. (a) G1 and S phase regulators Cdc2-Puc1 (solid black), Cdc2-Cig2 (dash-dotted) and Rum1 (solid red); (b) Negative regulatory kinase Wee1(dash-dotted), Mik1(solid black) and the positive regulator Cdc25p (solid red); (c) PP1 (dash-dotted), preMPF (solid black) and MPF (solid red) ; (d) Mitotic exit and M-G1 transition regulators APCp-Slp1(dash-dotted), Separase (solid black), APCp-Ste9 (solid red), PP2 (solid blue) and Mass.

network components and kinetic rate constants? Several independent analysis formulated in this study answers this question. Sensitivity analysis was performed for all 16 synthesis rate constants among the 128 parameters of this model which would reflect the influence of synthesis level regulation on the overall phenotypic characteristics. As a first step, the 16 synthesis rate constants were perturbed one at a time and the maximum magnitude of the perturbation, beyond which a steady state cyclic response vanishes, was determined. Table 1 summarizes the perturbation limits, within which the cell cycle network shows oscillatory response. When the synthesis rate constants were doubled, one at a time, a steady state cyclic response was observed for 15 of the 16 regulatory proteins. The only exception was Cdc2 for which the viable oscillatory response was observed until an increase of 93% in its synthesis rate constant from its nominal value. To evaluate the effect of decreasing activity, when the synthesis rate constant was individually set to zero, 10 of the 16 background showed viable phenotype. From this it is evident that these six regulators absolutely essential for the cell cycle regulation of *S. pombe*. Setting the synthesis rate constant for a particular protein to zero would mean absence of that specific regulator, therefore a single deletion mutant. Table 1 has the list of this six regulatory proteins which also indicates the exact percentage at which the oscillatory dynamics vanishes for all these six regulators. The cell can tolerate only a 3% decrease in the rate constants of Cdc13 and Cdc25, and a 31% decrease in case of APC synthesis. Interestingly, it is these six regulators in their absence resulted in inviable phenotype of the *S. pombe* cell cycle, when single deletion mutant simulations were performed in our previous work [17]. This is one of the remarkable observations made from this study.

While the cell cycle network of *S. pombe* demonstrates robustness to large perturbations in synthesis rate constants when taken one at a time, a different picture emerged when all the rate constants were perturbed simultaneously. A preliminary analysis done to understand the simultaneous perturbations in the synthesis rate constants showed that, when uniformly increased all together (all sixteen synthesis rate constant) by a fold (i.e. nominal set+100% of nominal set) from their respective nominal value the simulation results exhibited viable phenotype with approximately 200 minutes of cycle time which was within the chosen viability range (120-240 minutes). While trying to find the effect of decrease in the parameter values from the nominal set of rate constants, oscillations disappeared between 12-13%. 12% (nominal set - 12% of nominal set) decrease in the parameter set from their respective nominal values yielded steady state cycles with cycle period of approximately 160 minutes.

Following which a multi-parametric global sensitivity analysis was performed by randomly perturbing all 16 synthesis rate constants within a range of -100 to+100% from their respective nominal values. This enabled controlled change of chosen set of parameters within a defined range. A total of 7629 simulations were performed, each with a different set of synthesis rate constants. The simulated dataset (Figure 3) can be categorized into 13% that showed a cyclic steady state response within the viable range of 2-4 hours of cycle time chosen, 72% that showed a monotonic response, 13% that showed a cyclic steady state but with a period both less than 2 hours or greater than 4 hours and 2% that showed a non steady state cyclic behavior. While simulating, for each new parameter set generated (equation 1), periodic sensitivity coefficient (equation 2) was estimated only for the combinations that exhibited cyclic steady state within the assigned viable time limit of 120 to 240 minutes.

Regulator	Nominal Synthesis Rate	Upper limit (in Percentage)	Lower limit (in Percentage)
Cdc2	0.1	28	80
Cdc13	0.2	-	94
Cdc25	0.02	-	10
PP1	0.2	-	16
APC	0.02	-	11
Slp1	0.1	-	55

Table 1: Local sensitivity analysis for synthesis rate constants.

The average sensitivity coefficient for the respective synthesis rate constant and their standard deviation were also quantified (Table 2). Figure 4 shows the mean and standard deviation of the periodic sensitivity coefficients. Further, the synthesis rate constants were ranked based on the standard deviation of the sensitivity coefficients obtained (Table 2). Based on the rank order one can determine the regulator that has more influence on the regulation overall cell cycle period (Table 2 column 5). PP2 with rank 16 does not seem to affect the cell cycle period. Securin has the highest influence on cell cycle time followed by Cig2, Cdc2, Separase, Puc1 and Wee1. PP2 the M-G1 regulatory phosphatase is the one that does not have any significant influence in controlling cell cycle time with least standard deviation.

Robustness can help a theorist to recognize the correct model. Our local sensitivity analysis demonstrated that while the individual regulators were perturbed one at a time the model exhibited robust behavior. On the contrary when all sixteen parameters were perturbed

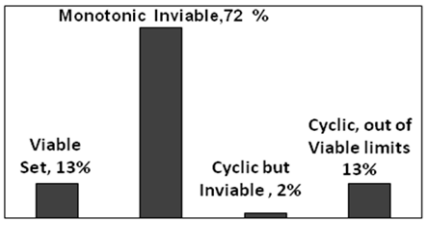

Figure 3: The simulated dataset of multi-parametric global sensitivity analysis. The dataset can be classified into, (i) 13% that showed a cyclic steady state response within the viable range of 2-4 hours of cycle time chosen; (ii) 72% that showed a monotonic response; (iii) 2% that showed a non steady state cyclic behavior and (iv) 13% that showed a cyclic steady state but with a period both less than 2 hours or greater than 4 hours.

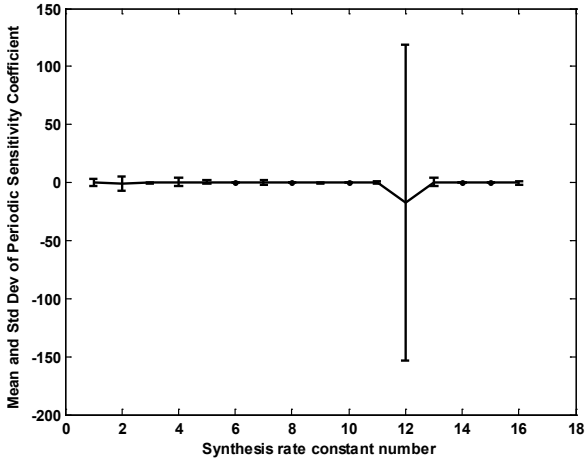

Figure 4: Periodic sensitivity of synthesis rate constants. Mean values and standard deviation of synthesis rate constants. Sensitivities were determined by -100% to +100% random variation of synthesis rates from its nominal values used in the model.

Synthesis	Synthesis Rate No	Synthesis rate constant (nominal)	$\langle S_\tau \rangle \pm \sigma^a$ (for 100% perturbation from its nominal value)	Rank[b]
S_Puc1	1	0.08	-0.0118 ± 2.7230	5
S_Cig2	2	0.1	-1.0677 ± 5.9802	2
S_Cdc13	3	0.2	-0.2842 ± 0.4951	11
S_Cdc2	4	0.1	0.4758 ± 3.8454	3
S_Rum1	5	0.05	0.2789 ± 1.5105	7
S_Mik1	6	0.01	0.0297 ± 0.3950	12
S_Wee1	7	0.02	-0.2168 ± 1.8685	6
S_Cdc25	8	0.02	0.0641 ± 0.3746	13
S_PP1	9	0.2	-0.2980 ± 0.5485	10
S_APC	10	0.02	0.0786 ± 0.2942	15
S_Slp1	11	0.1	-0.0590 ± 0.8842	9
S_Securin	12	0.08	-17.4183 ± 135.9810	1
S_Separase	13	0.02	0.5208 ± 3.7450	4
S_Ste9	14	0.02	0.0045 ± 0.3129	14
S_PP2	15	0.02	0.0988 ± 0.2921	16
S_I	16	0.06	-0.2662 ± 1.2718	8

[a]Indicates mean of periodic sensitivity coefficient and the standard deviation
[b]Based on average periodic sensitivity coefficient observed in global sensitivity analysis

Table 2: Global periodic sensitivity analysis.

only 30% of the parameters conferred cyclic steady state only 13% qualified to be yield viable phenotypic characteristics. This suggested that the system exhibits robust behavior for slight disturbance, such as the loss of function of one certain regulators, however when multiple regulators are affected the systems loses its inherent robust behavior. These results indicate that only one fifth of the perturbations yielded acceptable cell cycle behavior. While the single parameter perturbation show a robust behavior, the simultaneous perturbation analysis reveals that the acceptable cyclic behavior is lost in nearly 80% of parameter realizations. Thus, the sensitivity analysis indicates that the system is robust for single perturbations and fragile to multiple perturbations at the same time.

To further explore, the parametric robustness, a two-parameter sensitivity analysis was performed wherein any two synthesis rate constants were varied within their viable range obtained from local parameter sensitivity analysis while keeping all the other parameters at their nominal value. Their impact on the acceptable cyclic behavior was evaluated and classified as it was done for the global parameter sensitivity analysis. The parameter clusters that resulted in either viable or inviable responses were not separable and overlap each other (Results not shown). This indicates that the complexity is brought about by the interactions rather than the individual regulators. Thus, the two-parameter sensitivity analysis results can be correlated with the results of global periodic sensitivity analysis.

Systematic analysis for core cell cycle regulator identification and its implications

In order to understand the effect of regulatory components on the phenotypic characteristics a systematic network component deletion and addition analysis was performed. The questions that we try to answer through this systematic analysis are the following. Is it possible to predict the minimum set of regulators required to maintain *S. pombe* cell cycle regulation through *in silico* model? Can we predict the robust core regulatory network beyond which the cell exhibits fragile phenotype? The answer is yes and through systematic deletion analysis the minimum regulators that are necessary for keeping the cell cycle intact were identified to be Slp1, Cdc2, Cdc13, PP1, APC and Cdc25 along with either Mik1 or Wee1. These are the minimum regulators that are essential to keep the oscillatory cell cycle response intact. In the current study these six regulators along with wither Mik1 or Wee1 is referred as 6R+Mik1/6R +Wee1. Numerical simulation results indicated that the cell can withstand the deletion of all other regulators (Securin, Separase, PP2, I, Rum1, Ste9, Puc1, Cig2). In the absence of these regulators, in the 6R+Mik1 background oscillatory response with approximately 140 minutes of cycle time was observed. In the absence of these regulators, 6R+Wee1 combination resulted in oscillatory response with approximately 127 minutes of cycle time. Both of these responses were found to be within the viable limit of 120-240 minutes observed in experimental studies.

Furthermore, is it possible to trace the evolution of multiple regulators that participates in cell cycle regulation? There have been several earlier attempts [11,18]. Through systematic addition of the regulators, possible emergence of the regulators and their respective function in cell cycle during evolution is hypothesized. An iterative addition of all the other regulators (Securin, Separase, PP2, I, Rum1, Ste9, Puc1, Cig2) of the network to 6R+Mik1/Wee1 phenotype was performed and the cycle time close to fission yeast WT cycle time was chosen as the next best set or the next set of regulator that would have evolved. This process was repeated until the last regulator was added. The results and the mutant phenotypes are listed in the supplementary information (Tables S1 and S2). From the results tabulated in the supplementary information the possible evolutionary emergence of regulators with specialized function were deduced. Thus, this study also led to derive several independent insights.

Discussion

Diverse quantitative analysis was carried out to understand the roles of individual regulators, network structure of fission yeast *S. pombe* cell cycle regulatory network. An existing mathematical model developed by us was utilized for this investigation [17]. The nominal parameters sets of this model and the regulatory network structure that closely captures the wild type cell cycle regulation in fission yeast were systematically perturbed to observe their influence over the phenotypic characteristics such as viability and cell cycle time and to draw general conclusions. The sensitivity analysis provided insights into the influence of the various model parameters on the performance of the governing

regulatory network. Large scale theoretical studies that investigate the robustness characteristics through parameter analysis are very recent development in the area of the cell cycle regulation [13,14,19,20]. The present analysis focused on the influence of the synthesis rate constant of the regulators and their implication on the end phenotype of the fission yeast cell cycle. When only a single parameter was perturbed at an instance, the cell could withstand a wide range of variation from its nominal value. However, when a set of (multiple) parameters were perturbed at an instance, only certain combinations of parameter values yielded viable characteristics. This clearly demonstrated that it is the combinatorial effect of these regulators that determines the overall network behavior, thus the phenotypic characteristics.

Mean periodic sensitivity coefficients estimated through a global periodic sensitivity analysis indicated that Securin, Cig2, Cdc2, and Separase (descending order) were the when compared to all the other regulators of the model. It is well known that these essential regulators play crucial role in governing proper DNA replication and chromosome segregation during each growth and division cycle of fission yeast [2]. The synthesis rate constant of Securin, determines the stoichiometric availability of Separase, the regulator essential for the physical separation of the chromosomes between the daughter cells. Since Cdc2 is the only cyclin dependent kinase to activate all cyclins of *S. pombe*, its availability is important in establishing and maintaining the appropriate activity level of MPF. As a component of MPF which controls the activity of several positive negative regulators Cdc2 regulates different phase specific growth characteristics. It contributes in determining the cycle period by activating its own destruction by means of anaphase promoting complex (APCp-Slp1). Further, the synthesis rate of Slp1 was also found to greatly influence the cycle time. It is interesting to note that the rate constants that were found to be highly sensitive, also correspond to the regulatory proteins that play a crucial role in providing the essential threshold effect and time delay for proper growth, replication and segregation of genetic material.

Regulators such as PP1 and Ste9 show low periodic sensitivity and the cell cycle operates even in their individual absences. A notable exception is Cdc25, which despite of its low periodic sensitivity, is lethal when absent. The observations and the ranking obtained from global sensitivity analysis are relative measures of the robustness or sensitivity of this system because of the highly interconnected nature of this complex network. The response observed is also a cumulative sum of the influence of all the sixteen synthesis rate constants during a particular simulation. Yet it demonstrates the influence of synthesis level regulation on maintaining the cell viability and cell cycle period. An independent study along similar lines concludes that protein degradation is crucial to generate the oscillatory behavior of the cell cycle and the synthesis is responsible for controlling period for budding yeast cell cycle regulation [14]. The present study indicates that the synthesis level regulation has influence on both periodicity and oscillations.

The systematic identification of core regulators of *S. pombe* provides a few interesting insights and perspective regarding the minimal regulators which are essential to keep the fission yeast cells viable and the possible emergence of evolutionarily control mechanism of simple eukaryotic yeast like fission yeast *S. pombe*. Systematic deletion analysis suggests Slp1, Cdc2, Cdc13, PP1, APC and Cdc25 along with either Mik1 or Wee1 to be the minimum regulators that are necessary for keeping the cell cycle intact. These core regulators identified to be essential for viability is also universally conserved across eukaryotes [2]. There are early mathematical models available that demonstrated the cell cycle characteristics with these exact minimal set to regulators. For example the model by Srividhya et al. [21] demonstrates almost all the crucial cell cycle properties with a minimum of five regulators (Cdk, Cyclin, Wee1, Cdc25, and APC). This observation of the present analysis is similar to the experimental results by Coudrouse and Nurse [22]. Their study points out that Cdc2 and Cdc13 are the minimum essential regulators which is sufficient for the survival of the *S. pombe* cells and to drive the cell cycle regulation [22]. There is also a mathematical model in support for this synthetic biology experiment [10]. Similar efforts have been made by several others to identify the core essential regulators. Decottignies et al. [23] have performed PCR based gene deletion study. There are early mathematical models that discusses about the minimum core regulation required for cell cycle to progress [18]. It is also interesting to note that these regulators Slp1, Cdc2, Cdc13, PP1, APC and Cdc25 if individually deleted (single deletion mutant) led to inviable phenotype (our previous study, Supplementary Table S2 in Anbumathi et al.) [17]. In the current study these are the most sensitive regulators (Table 1). Thus, along with Cdc2, Cdc13, Cdc25, PP1 and APC, Wee1 or Mik1 contributes to the growth and cell division regulation of *S. pombe*. The negative regulators (Wee1/Mik1) provide the essential time necessary for the cells to grow and mature before they enter mitosis by maintaining Cdc2 at its lower concentrations. This core network has all the essential components required to bring in essential switch like transitions of the cell cycle through direct positive feedback (Cdc2, Cdc13, Cdc25), through antagonistic hysteresis type indirect positive feedback effect (Cdc2, Cdc13 and Wee1/Mik1) and the ubiquitin mediated negative feedback (APC and Slp1) loop that could terminate the cell cycle at the end of division. The viability and physiological properties of an organism with just the above mentioned regulators to drive the cell cycle can be validated through synthetic biology experiments.

Apart from indentifying the core regulators for the cells viability the present analysis can also be utilized to trace the evolution of cell cycle regulators in simple eukaryote like *S. pombe*. Model scenarios for evolution of the eukaryotic cell cycle evolution are available in literature. Novak et al. [18] based on Nasmyth [24] primordial theory of cell cycle evolution have developed and predicted model scenarios for evolution of the eukaryotic cell cycle. Reverse engineering study by Csikász-Nagy et al. [11] also demonstrate the bottom up design principles through addition of feedback based layers to the minimal core regulator of the cell cycle. From the results tabulated in the supplementary information it is evident that the cell size remains small (wee) in 6R+ Mik1 background (Table S2) until the last step where Wee1 is added. There are ample experimental evidences that confirm the role of Wee1 in cell size regulation [25-27]. Mik1 and Wee1 are both inhibitory regulatory kinases that regulate MPF during early G2 phase and G2-M phase transition, respectively. Wee1ΔMik1Δ double deletion mutant shows synthetically lethal phenotype [28]. Mutant simulations of the model developed reflect this synthetic lethality *in silico*. Further, their protein sequence similarity match of 48% indicates that they are conserved proteins [28]. Mik1 is present in Chromosome II and Wee1 in Chromosome III (http://old.genedb.org/genedb/pombe/). From these evidences we can speculate that one of these inhibitory kinases might have evolved from gene duplication events and over prolonged period the size control might have been associated with Wee1 gene. Additional pairwise (LALIGN) and multiple sequence (CLUSTAL W) alignment performed to test this hypothesis indicated 39.7% identity in 325 amino acid overlap between these two regulators and they were found to align close to each other in same branch (Figure 5). Nevertheless, this preliminary hypothesis based on the insights drawn from these

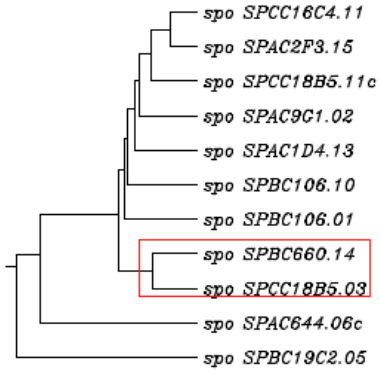

Figure 5: Rooted phylognetic tree representation of multiple sequence alignment carried out within the serine, tyrosine kinase superfamily of *S. pombe*.

in silico analysis and information available in literature needs further experimental investigations. Carefully designed genome scale *in silico* analysis and experiments can assist in validating this hypothesis.

Conclusions

The present study, through a series of simple systematic analysis investigated the sensitivity of the core cell cycle regulatory network of fission yeast *S. pombe*. Results of a systematic local and the global perturbation of synthesis level regulation indicates that the cell cycle time is robust to perturbation of single regulators but fragile to simultaneous perturbation of the multiple regulators. Systematic regulator deletion and analysis has provided lights on the core regulatory network which comprises Slp1, Cdc2, Cdc13, PP1, APC, Cdc25 along with either Mik1 or Wee1. These are the essential intrinsic regulators which are required for independent cell cycle regulation. Multiple layers of complex regulators could have evolved over this fragile, yet essential core regulator of cell cycle during eukaryotic evolution which could have led to complex interaction networks. The key predictions, (i) seven core regulatory components Slp1, Cdc2, Cdc13, PP1, APC, Cdc25 along with either Mik1 or Wee1 are sufficient to build a synthetic eukaryote with an independent growth and division cycles; (ii) either one of the G2 regulatory kinases Wee1 or Mik1 could have emerged through whole chromosome duplication events during evolution; can be tested experimentally to arrive with a conclusive proof. Thus, individual results of these analysis were utilized to explore the interplay between the sensitivity (robustness or fragility) and the complexity of the evolved molecular network used to model fission yeast *S. pombe* cell cycle regulation. Comparing to the actual system which has thousands of regulatory components at gene, protein and metabolic level the protein interaction network utilized in this study is considerably small, yet the insights derived sheds light on some of the fundamental characteristics of living systems.

Methods

The present study through several independent analysis investigates diverse network characteristics of the existing fission yeast *S. pombe* cell cycle model. Detailed description of the molecular details, model equations and the nominal parameter sets considered in this study can be found in Anbuamthi et al. [17]. Various procures followed to perform these independent analysis are documented below.

Sensitivity analysis

Three different sensitivity analyses were performed. Of the 128 model parameters, synthesis rate constants of the sixteen regulators of the model were chosen to study their influence on the overall cell cycle regulation phenotypic characteristics. In the preliminary local sensitivity analysis, individual rate constant was perturbed one at a time within the range from -100% to +100% of their respective nominal value utilized to model the wild type. One fold decrease and increase from the respective nominal parameter was determined as the minimum and the maximum accessible range for this local sensitivity analysis. Oscillatory or cyclic behavior was deemed viable and indication of balanced growth and division. All the sixteen parameters were individually, decreased or increased to note the resulting percentage at which oscillations failed to occur. Finally, a global periodic sensitivity analysis was formulated to quantify the influence of the simultaneous random perturbations in all synthesis rate constants on the cell cycle period. In this case, a steady state cyclic response with a period between 2-4 hours was deemed viable. The metric used to quantify the periodic sensitivity coefficient captures the change in the cell cycle period upon change in the parameter P is a modified form described by Stelling et al. [29] for circadian rhythms. For each simulation the new parameter set was selected randomly within -100% to +100% (i.e. within one fold change in the positive and negative range from its nominal value) of its nominal value used to simulate wild type model. The following equation describes how the set of sixteen synthetic rate constants were generated for each simulation.

$$P_{RP} = P_N \pm rand \sum_{0}^{100\%} P_N \quad (1)$$

where, P_{RP} is the new set of synthesis rate constants generated within -100% to +100% of its nominal synthetic rate constant P_N through random selection using Matlab 'rand' function. The model was simulated for each new parameter set of P_{RP} generated (equation 1) and the resulting dynamics was analyzed for its emerging dynamics. The periodic sensitivity was quantified only for the set of parameters that resulted in steady state oscillatory response after 4500 minutes of simulation. The peaking of MPF was utilized to estimate the new cycle period τ_{RP}. The periodic sensitivity coefficient for oscillatory dynamics that falls under the viable limits of 120-240 minutes of cell cycle time was estimated by utilizing the following metric,

$$S_\tau = \frac{(\tau_{RP} - \tau_N)/\tau_N}{(P_{RP} - P_N)/P_N} \quad (2)$$

where, S_τ is the periodic sensitivity coefficient; P is the synthesis rate constant and 'τ' is the respective cell cycle time; the subscripts *RP* and *N* respectively indicates the random perturbation and nominal response.

Identification of the core regulators

The goal of devising this analysis was to detect the minimal regulators required to drive fission yeast *S. pombe* cell cycle regulation from the network shown (Figure 1). To achieve the same a systematic deletion analysis was performed to indentify the robust core cell cycle regulators of the model considered in this study. Any perturbation to this core would lead to fragile inviable phenotype. The regulator combination that resulted in oscillatory response was considered to a measure of viability. The later part of this study explored the possible evolutionary path of the robust outer layer. For this we assumed that the preference was to stay viable and hypothesized that the combination that gave cycle time closer to the wild type cycle time was preferred over the rest. Both these regulator deletion, addition analysis involved

only modification at regulator level information of the network and the parameters utilized in the wild type model were not tinkered. In all of these analysis disappearances of oscillations or loss of cyclic behavior is assumed to produce inviable cells that have lost the basic characteristics of growth and division driven by this network.

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