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Abstract: Ostreococcus tauri is the smallest free-living unicellular organism with one copy of each core cell cycle genes in its genome. There is a growing interest in this green algae due to its evolutionary origin. Since O. tauri is diverged early in the green lineage, relatively close to the ancestral eukaryotic cell, it might hold a key phylogenetic position in the eukaryotic tree of life. In this study, we focus on the regulatory network of its cell division cycle. We propose a mathematical modelling framework to integrate the existing knowledge of cell cycle network of O. tauri. We observe that feedback loop regulation of both G1/S and G2/M transitions in O. tauri is conserved, which can make the transition bistable. This is essential to make the transition irreversible as shown in other eukaryotic organisms. By performing sequence analysis, we also predict the presence of the Greatwall/PP2A pathway in the cell cycle of O. tauri. Since O. tauri cell cycle machinery is conserved, the exploration of the dynamical characteristic of the cell division cycle will help in further understanding the regulation of cell cycle in higher eukaryotes.

***Cover Letter**

Dear Hiroshi Ezura,

Thank you for considering our paper entitled "*Systems-level feedback regulation of cell cycle transitions in Ostreococcus tauri*" for publication in Plant Physiology and Biochemistry. We thank the reviewers for their positive opinion and useful comments, which helped us to improve the quality of the manuscript. Our point-by-point replies to the reviewers' comments see in the file called "Response to Reviewers".

We hope that our manuscript in this revised form will be acceptable Plant Physiology and Biochemistry.

Yours sincerely,

Orsolya Kapuy

Our point-by-point replies to the reviewers' comments are the followings:

REVIEWER

Reviewer #1: Manuscript „Systems-level feedback regulation of cell cycle transitions in Ostreococcus tauri" presents an interesting set of data on modeling the cell cycle of green alga Ostreococcus tauri stemming from the known cell cycle regulators and their behavior during the cell cycle of the organism. The model fits the experimental data presented so far and brings forward some regulatory feedback relationships that were not obvious from the data alone. Moreover from bioinformatics data, there are some hints to other cell cycle regulators possibly involved in the cell cycle regulation of this alga. The manuscript presents a very interesting layout and brings forward several interesting hypotheses that would require further experiments. I generally like the manuscript since it is doing a decent job in explaining the models and different scenarios during the cell cycle. The reader is guided throughout the modeling and its predictions making drawing the conclusions simple
.
I have several mostly minor comments:
1)Throughout the manuscript it is not entirely clear to me what is according to the authors the main advantage of their model. Based on their models, its evolutionary position and the cell cycle gene components it looks like on the threshold between plant and other kingdoms. The cell cycle organization seems mammalian with plant specific features (presence of plant specific CDKB). Is the species more a mammalian model, a plant model or a unique organism able to be both? I think it would be interesting to disscuss.

We agree with the reviewer that it would be interesting to discuss about whether O. tauri is similar to mammalian models or plant ones. Therefore the Conclusion was extended with the following paragraph.
The purpose of our research was originally to analyse the cell cycle machinery of O. tauri and demonstrate how the cell cycle regulation of this ancient species resembles the much more complex plant ones. With building up a mathematical model we planned to prove that these unicellular algae can be reliably used to study plant cell cycle. To our surprise, however, we found that O. tauri cell cycle control network is much more similar to mammalian cell cycle regulation. In this respect, it seemed to even prefer the yeasts, therefore we emphasize that O. tauri might be a useful model organism to understand the mammalian cell cycle. Our results also suggest that cell cycle regulation of mammals from ancient unicellular eukaryotes has basically remained unchanged; meanwhile a multicellular plant was much more differentiated. We suppose that the complexity of mammalian cell cycle control network ensures the robustness of the organism at various external and internal signals; however the irreversible one-way directionality of cell cycle transitions, with the same accuracy, is already guaranteed in the ancient unicellular eukaryotic cell.

2)Along similar line, on page 4 l. 24-26 the authors argue about organismal complexity and cell cycle gene redundancy being an issue for cell cycle study. Is this valid for all eukaryotes or specifically for plants?

The sentence has been re-phrased.
Nevertheless the most well-known difficulties of cell cycle research are the organismal complexity of higher eukaryotes and redundancy in cell cycle genes is also observed in plants

3) Please be consistent in using past tense in Methods chapter. On several instances, future tense is used.

Methods chapter has been thoroughly revised and re-written using past tense.

4)P. 15, l. 19-20 CDKB is plant specific, it is not entirely clear yet if mitotic cyclin B/CDKB is specific for O. tauri or generally plant specific, please re-phrase

The sentence has been re-phrased.
Further, it is also known in higher eukaryotes that Cdc25 is enhanced, while Wee1 is inhibited by the mitotic CDK/Cyclin complex dependent Tyr-phosphorylation.

5)P. 15, l. 31-34 When discussing PP2A it would be interesting to relate to the phosphorylation sites identified by bioinformatics and discussed later on in the MS.

The paragraph has been re-written by extending it with the bioinformatic detection of CDK-dependent phosphorylation sites on PP2.
By using bioinformatic analysis 40 Ser and 22 Thr residues were identified on PP2A and one of the serines seemed to be a potential CDK-phosphorylation site (Table 3). This result suggests that PP2A might be directly regulated by the active CDK/cyclin complex.

6)P. 15, l. 48-49 Please replace the numeral 11 with proper reference

The reference mentioned by the reviewer has been fixed.

7)P. 15, l. 51-52 "The mitotic kinase activity…" maybe rather "The mitotic kinase remains Tyr-phosphorylated with low kinase activity…."

The text has been corrected.

Reviewer #2: This manuscript reports a modeling study of the control of the cellular cycle on a particularly eukaryotic organism: the green microalgae Ostreococcus tauri. The fully sequenced genome of this algae is very compact and has the important advantage of having virtually no multiple copies of the different genes. This is particularly true for genes whose products control the cell cycle, CDKs, Cyclins and others. The representation of the cell division cycle regulation network is based on a mathematical model, published annotations of potentially involved genes and published experimental results. In addition, an in-depth annotation identified the partners of the Greatwall/PP2A pathway of the cell cycle of O. tauri. This work seems to be based on solid modelling bases and uses the published experimental data appropriately. However, as I am not a modeling specialist and do not know the mathematical tools used, it is difficult for me to give an expertise on the mathematical construction of the model. However, the use of all published annotation or functional data is adequate.

Thank you so much the positive comments.

Highlights

- presenting a mathematical model to explain the cell cycle regulation of *O. tauri*
- both G1/S and G2/M transitions are controlled by double negative feedback loops
- the presence of PP2A, GWL, ENSA and/or ARPP19 is predicted in *O. tauri*

Systems-level feedback regulation of cell cycle transitions in *Ostreococcus tauri*

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Keywords: *Ostreococcus tauri*, cell cycle, greatwall, mathematical modelling, systems biology

Abbreviations: GWL: Greatwall, ENSA: Endosulphine

Abstract

Ostreococcus tauri is the smallest free-living unicellular organism with one copy of each core cell cycle genes in its genome. There is a growing interest in this green algae due to its evolutionary origin. Since *O. tauri* is diverged early in the green lineage, relatively close to the ancestral eukaryotic cell, it might hold a key phylogenetic position in the eukaryotic tree of life. In this study, we focus on the regulatory network of its cell division cycle. We propose a mathematical modelling framework to integrate the existing knowledge of cell cycle network of *O. tauri*. We observe that feedback loop regulation of both G1/S and G2/M transitions in *O. tauri* is conserved, which can make the transition bistable. This is essential to make the transition irreversible as shown in other eukaryotic organisms. By performing sequence analysis, we also predict the presence of the Greatwall/PP2A pathway in the cell cycle of *O. tauri*. Since *O. tauri* cell cycle machinery is conserved, the exploration of the dynamical characteristic of the cell division cycle will help in further understanding the regulation of cell cycle in higher eukaryotes.

1. Introduction

Cell growth and division are basic properties of all living organisms and are highly conserved through evolution. The cell cycle machinery ensures the alternation of DNA replication (S phase) and chromosome segregation (M phase) (Morgan, 2007). The molecular players involved in cell division cycle have been widely studied in various eukaryotes, including humans, plants and yeasts. Cell cycle regulation is basically controlled by the kinase activity of the family of CDK/cyclin heterodimers (Morgan, 2007). Although CDK/cyclin complexes are existing in all eukaryotic lineages, some regulatory modules of cell division cycle have evolved differently, such as the Rb pathway present both in animals and plants, but absent in yeasts (Cross et al., 2011). Nevertheless the most well-known difficulties of cell cycle research are the organismal complexity of higher eukaryotes and redundancy in cell cycle genes is also observed in plants (De Veylder et al., 2007, Dewitte & Murray, 2003). Therefore, there is growing need to employ simpler eukaryotic model organisms such as *Ostreococcus tauri* to study the cell cycle regulation (van Ooijen et al., 2012, Claude Courties, 1998).

O. tauri is the smallest free-living unicellular eukaryotic organism with a diameter of less than 1 μm. Its cellular organization is simple with one copy of its important organelles, such as mitochondrion and chloroplast (Robbens et al., 2005). Its full genome, distributed among 18 chromosomes, has already been fully sequenced (Derelle et al., 2006). The evolutionary origin of *O. tauri* is of significant interest (van Ooijen et al., 2012, Claude Courties, 1998). Since this green alga diverged early in the green lineage, relatively close to the ancestral eukaryotic cell, it might hold a key phylogenetic position in the eukaryotic tree of life.

It is well-known that *O. tauri* cells divide by binary fission. The regulatory principles underlying its cell cycle control can be explored as some of the regulators have been

identified (Corellou et al., 2005, Farinas et al., 2006). Although annotation of most of *O. tauri* cell cycle genes showed high similarity to plant cell cycle genes, interestingly some genes show high similarity to its animal homologue (Robbens et al., 2005). The genome-wide analysis of core cell cycle genes in *O. tauri* revealed that most of them are present only in one copy. *O. tauri* has one homolog of CDKA, CDKB and CDKD that are involved in cell division control. Besides the organism has the minimum set of cyclins (i.e. CycA, CycB, CycD and CycH) (Robbens et al., 2005). Rb- and APC-regulated G1/S transition and mitotic exit, respectively, have been reported (e.g. APC, CDH1, CDC20, Rb and E2F) (Robbens et al., 2005). The regulators of G2/M transition in metazoans and yeasts namely Wee1 kinase and Cdc25 phosphatase are also present in *O. tauri* (Robbens et al., 2005). Although *Arabidopsis* has many CDK inhibitor (CKI) Kip-related proteins there are no such related CDK inhibitors that can be found in *O. tauri* by sequence similarity searches (Robbens et al., 2005).

Cell division frequently occurs at specific time of the day suggesting that the photoperiodic control of this process is mediated through the circadian clock. It is shown that the core cell cycle genes (i.e. cyclins, CDKs) are under circadian control in *O. tauri* (Moulager et al., 2010, Corellou et al., 2005, Farinas et al., 2006). At physiological conditions, the timing of cell cycle entry was not observed prior to 6 hours after dawn (Moulager et al., 2010). Although the crucial elements of cell division cycle are transcribed independently of the amount of light in G1 phase, the G1/S transition occurs in a light-dependent manner via cAMP. The level of cAMP increased immediately after light on and had a transient activity peak to promote Cyclin A synthesis before S phase was detected (Moulager et al., 2010). Both down-regulation of Rb and overexpression of Cyclin A triggered cell cycle entry under limiting light conditions suggesting that light-dependent control of cell division occurs via Rb pathway at G1/S (Moulager et al., 2010). Recent studies have used *O. tauri* as a model system to

understand the basic underlying mechanisms of plant circadian clock, as well (Pfeuty et al., 2012, Thommen et al., 2012).

In this study, we propose a theoretical framework to investigate *O. tauri* cell cycle. Mathematical model of G1/S and G2/M transitions in *O. tauri* are developed that brings together various experimental data. We observe that double negative and positive feedback loop design present in these transitions is conserved, which can make the transition bistable. We propose that this dynamical feature is the key to make the transition irreversible in *O. tauri*. Further, by sequence analysis, we also predict the presence of the Greatwall/PP2A pathway in the cell cycle of *O. tauri* as observed in other organisms. Our results suggest that the cell cycle regulatory network of this unicellular alga and mammalian cells shows high similarities. Our analysis highlights that *O. tauri* can be used as an alternative eukaryotic model organism to further explore the dynamics of cell cycle division network.

2. Methods

2.1. Mathematical model description

The regulatory network of *O. tauri* cell cycle was translated into a set of ordinary differential equation (ODE) that described how each component concentration/activity in the network changes with time. A generic differential equation depicting the temporal changes of a regulatory component is composed of two parts: production and consumption terms. The production can be given by protein synthesis and/or an activation term, while the consumption can be specified by protein degradation and/or inactivation term. Usually synthesis, degradation, binding and dissociation reactions are described by mass action kinetics, whereas protein activity can be described either by mass action or Michaelis-Menten kinetics. The equations were solved numerically with *XPP-AUT*. This program is freely available from G. Bard Ermentrout, Department of Mathematics, University of Pittsburgh (http://www.math.pitt.edu/~bard/xpp/xpp.html).

The time evolution of the protein activity and/or level of the key components were studied by implementing time courses. The time courses have been calculated by numerical integration of the full set of our differential equation system of contained by our model.

To understand characteristic dynamical features of the regulatory network and its dependence on its parameter values, two-dimensional phase plane analysis were carried out. In two-dimensional phase plane analysis the two slowest variables were followed, while fast variables were separated and assumed to be already in steady state. The kinetic behaviours of the differential equations were visualized graphically by balance curves on phase plane portrait. The balance curve of a protein level or activity denotes where the rate of the protein production and consumption terms are balanced. This method represents the observable physiological states of the cell cycle regulatory system. The intersections between two

balance curves are called equilibrium points: here the system has steady-state solutions (H., 1994).

Plotting signal response curves were used to understand the dynamic changes in the nonlinear system as a function of a specific parameter (Tyson et al., 2003). A single variable was chosen from the complex regulatory system which was supposed to characterize all interacting proteins in the network. Moreover, the selected parameter could represent the main effect involved in these relations. The behaviour of the system were graphically analysed on signal response curves. The stable solutions with solid lines, the unstable solutions with dashed lines are depicted on signal response curves (H., 1994).

All the simulations presented in the text were based on *XPP* codes given in the Appendix which contains ODEs. The rate constants (k) have the dimension of min^{-1} and Michaelis constants (J) are dimensionless. The proteins levels/activities are given in arbitrary units (a.u.).

2.2. The basics of bioinformatical analysis

Bioinformatics searches for candidate GWL, PP2A, ENSA and ARPP-19 homolog sequences were performed using Standard Protein Basic Local Alignment Search Tool (Blast) (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&DBSEARCH=true&QUERY=&SUBJECTS=) at NCBI, using default parameters and settings.

For comparing various sequences Align Sequences Protein Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins&PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&DATABASE=n/a&QUERY=&SUBJECTS=) at NCBI was applied, using default parameters and settings.

To identify the gene sequences in *O. tauri* KEGG GENOME: *Ostreococcus tauri* was used (http://www.genome.jp/kegg-bin/show_organism?menu_type=pathway_maps&org=ota).

To search for putative GWL- and CDK-dependent phosphorylation sites on PP2A, ENSA and ARPP19 NetPhos 3.1 was used. Potential Ser and Thr phosphorylation sites were depicted and phosphorylation sites with score higher than 0.5 were collected separately.

3. Results

3.1. The regulatory network of cell cycle G1/S transition in *Ostreococcus tauri*

We explored the dynamical characteristic of cell cycle network of *O. tauri* using mathematical modelling approach. Focusing on G1/S and G2/M transitions, we built up a wiring diagram of the components of *O. tauri* cell cycle machinery based on the experimental data available in the literature (Figure 1A and 2A).

Cyclin A is transcribed in early G1 phase and forms a complex with CDKA. The overexpression of Cyclin A is shown to advance the timing of S phase entry (Moulager et al., 2010). Interestingly, the C-terminal part of Cyclin A contains the retinoblastoma (Rb) binding motif suggesting that CDKA/Cyclin A complex has an essential role in G1/S transition (Robbens et al., 2005). *O. tauri* genome has one copy of both Rb and E2F genes. Moulager et al. (2010) have shown that down-regulation of Rb induced early cell division (Moulager et al., 2010). In addition, CKIs could not be identified by sequence similarity searches in the *O. tauri* genome (Robbens et al., 2005). Since Rb is a well-known regulator of G1/S in various organisms, we consider that cellular commitment in G1 is controlled by Rb pathway in *O. tauri*, too (Figure 1A). We assume that CDKA/Cyclin A directly inhibits Rb by phosphorylation, meanwhile Rb might have a negative effect on CDKA/Cyclin A.

We suppose that E2F induces Cyclin A, although it has not proved yet that E2F is the key transcription factor of Cyclin A in *O. tauri*. The mRNA level of Cyclin A is expressed from dawn and it seems not depending on E2F for transcription (Moulager et al., 2010). However the protein level of Cyclin A shows a remarkable increase in G1 suggesting that translation of Cyclin A mRNA is under strict control. We suggest that E2F might be required to control the translation of Cyclin A mRNA indirectly. In our model for simplicity we assume that E2F has a direct positive effect on Cyclin A, meanwhile Rb inhibits E2F (Figure 1A).

In our model Rb binds to the transcription activator, E2F, to block the cell cycle entry via down-regulation of CDKA/Cyclin A, while the cell cycle kinase inhibits Rb generating a so called double negative feedback loop in the control network (Figure 1A).

Experimental data have shown that cell division itself could be light independent, although light was essential to induce cell growth. When *O. tauri* cells cross the commitment point in G1, the cell division occurs independently of light (Moulager et al., 2010). Moulager et al. (2010) showed that commitment is controlled by Rb pathway and that CDKA/Cyclin A plays key role in controlling cell cycle progression in G1 by regulating Rb phosphorylation and timing of S-phase entry (Moulager et al., 2010). We used a mathematical model to understand the dynamical characteristic of light-dependent cell cycle regulation in G1. We demonstrate that the irreversible cellular commitment can be controlled by the double negative feedback loop between CDKA/Cyclin A and Rb (Figure 1A).

3.2. G1/S transition in *O. tauri*: Rb pathway regulation by light intensity and duration

Moulager et al. (2010) showed that the translation of Cyclin A mRNA is controlled by light conditions in cAMP dependent manner (Moulager et al., 2010). We show that due to the double negative feedback loop between CDKA/Cyclin A and Rb, cAMP activity has to reach a critical threshold to turn on Cyclin A synthesis (Figure 1B). This feedback loop regulation makes the transition bistable with respect to cAMP activity. This suggests that critical light intensity and duration, which control cAMP activity, is required to promote S-phase entry and commitment to cell division in absence of light. In this scenario, high light intensity requires lesser duration to promote S-phase entry.

At physiological conditions the balance curves of Rb (see red line on Figure 1C) and CDKA/Cyclin A (see green line on Figure 1C) have three intersections with two stable steady states (see filled black circles on Figure 1C) separated by an unstable one (see open circle on

Figure 1C) suggesting a bistable characteristic for the control network. One stable state represents the G1 state with high level of Rb and low level of CDKA/Cyclin A, while the other state (i.e. CDKA/Cyclin A level is high, Rb is inactive) corresponds to S phase (Figure 1C). If level of Cyclin A gets overexpressed the CDKA/Cyclin A balance curve shifts upwards on the phase plane (see the green curve on Figure 1D), therefore G1 state disappears and the requirement of light and cAMP level is bypassed to enter S phase. The down-regulation of Rb also results in the loss of G1 steady state and an early S phase entry is observed, since the Rb balance curve moves downwards on the phase plane portrait (see the red curve on Figure 1E).

Our analysis highlights that an alteration in the bistable switch generated by CDKA/Cyclin A – Rb double negative feedback loop might allow an early S phase entry bypassing the light requirement in *O. tauri*.

3.3. The control of G2/M transition in *O. tauri*

Although CDKA is the mitotic kinase in higher plants CDKB also seems to play a crucial regulatory role in green alga cell cycle. CDKA activity remains constant and relatively low during cell cycle G1 progression and disappears at mitotic exit (Corellou et al., 2005, Farinas et al., 2006). CDKB forms a complex with Cyclin B in G1. CDKB/Cyclin B activity remains relatively low both in S and G2 phases, while it has a huge activity peak at G2/M transition (Corellou et al., 2005, Farinas et al., 2006). Both CDKB and Cyclin B disappear at M/G1 resulting in a fast drop of kinase activity at mitotic exit (Corellou et al., 2005, Farinas et al., 2006).

Although CDKB/Cyclin B is present both in S and G2 phases, the experimental data have shown that the complex gets inhibitory Tyr-phosphorylated while CDKA/Cyclin A is not inhibited by phosphorylation (Corellou et al., 2005, Farinas et al., 2006). Interestingly both

regulators of G2/M transition in metazoans and yeasts, i.e. Wee1 kinase and Cdc25 phosphatase can be found in *O. tauri* (Robbens et al., 2005, Corellou et al., 2005, Farinas et al., 2006, Khadaroo et al., 2004). It has been shown that Tyr-phosphorylated CDKB/Cyclin B becomes active only at mitotic entry by Cdc25-dependent dephosphorylation (Corellou et al., 2005, Farinas et al., 2006). We assume that Wee1 kinase has a negative effect on CDKB/Cyclin B by inhibitory Tyr-phosphorylation. Since it is well-known that both Cdc25 and Wee1 can be phosphorylated by the mitotic CDKB/Cyclin B complex in higher eukaryotes, which increases and decreases its activity, respectively; therefore we claim that these regulatory connections are also present in *O. tauri* cell cycle machinery (Figure 2A).

CDK controls eukaryotic cell cycle events by activating/inactivating its substrates through phosphorylation. Therefore, the regulatory network requires a phosphatase to dephosphorylate the above mentioned CDK targets. It has been recently shown in various eukaryotic organisms that the CDK counteracting phosphatase is PP2A-B55 (Lorca & Castro, 2013). PP2A-B55 can dephosphorylate key substrates of CDKB/Cyclin B, such as Cdc25 and Wee1 (Jeong & Yang, 2013) (Figure 2A). With Basic Local Alignment Search Tool from NCBI we could identify the serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B (NCBI-Gene ID: 9834125) gene in *O. tauri* genome, which showed 95% similarity to human PP2A-B55 sequence (Table 1). Therefore, we assume that this phosphatase might play a similar role in *O. tauri* as CDK counteracting phosphatase (Figure 2A). Besides, the existence of PP2A-B55 in *O. tauri* also suggests that it can be regulated by Cdk1 as shown in other organisms. Therefore, we have further explored the phosphatase regulation in this work.

3.4. The role of feedback loops in G2/M transition in *O. tauri*

Although the mitosis-inducer Cdc25 phosphatase is absent in plants, one copy of both Cdc25 and Wee1 can be found in *O. tauri* genome. Interestingly the green algae Cdc25 was able to

rescue the *Schizosaccahromyces pombe cdc25-22* conditional mutant and also promote the mitotic entry of prophase-arrested starfish oocytes (Khadaroo et al., 2004) suggesting that Cdc25 found in *O. tauri* might have an important role in controlling mitotic entry. In higher eukaryotes a well-known kinase that promotes Tyr-phosphorylation of mitotic kinase is Wee1. Further, it is also known in higher eukaryotes that Cdc25 is enhanced, while Wee1 is inhibited by the mitotic CDK/Cyclin complex dependent Tyr-phosphorylation. If we assume the presence of these above mentioned regulatory connections in *O. tauri* cell cycle machinery the mitotic kinase activity is driven by two feedback loops: via both CDKB/Cyclin B – Cdc25 positive and CDKB/Cyclin B – Wee1 double negative feedback loops (Figure 2A). By using bioinformatic analysis 40 Ser and 22 Thr residues were identified on PP2A and one of the serines seemed to be a potential CDK-phosphorylation site (Table 3). This result suggests that PP2A might be directly regulated by the active CDK/cyclin complex. We also suppose that the dephosphorylation of both Cdc25 and Wee1 is controlled by the CDK-counteracting phosphatase. This PP2A-depedent regulation generates additional double negative feedback loops in the mitotic entry regulation of *O. tauri*, namely between PP2A and CDKB/Cyclin B through Cdc25 (CDKB/Cyclin B ┤ PP2A ┤ Cdc25 -> CDKB/Cyclin B) and Wee1 (CDKB/Cyclin B ┤ PP2A -> Wee1 ┤ CDKB/Cyclin B) (Figure 2A).

The feedback loop regulation of CDK ensures that its activity does not increase until Cyclin B accumulates to reach a threshold and make the G2/M transition bistable. Cyclin B accumulation might be dependent on E2F once the cells have passed the commitment (Moulager et al., 2010). The mitotic kinase remains low and Tyr-phosphorylated during S and G2 phases (see the intersections of total level of CDKB/Cyclin B – the active CDKB/Cyclin B balance curves on Figure 2B), but its activity increases at mitotic entry drastically. We also show that S-phase block performed by HU addition in *O. tauri*, will shift the threshold to

activate CDK to higher concentration of Cyclin B, thereby block cells in S/G2 boundary (Figure 2C).

Corellou et al. (2005) have shown that in vitro Cdc25 addition resulted in a dose-dependent escalation of H1 kinase activity (Corellou et al., 2005, Farinas et al., 2006). We also observe that the threshold to activate CDK is sensitive to Cdc25 concentration and increase in Cdc25 concentration reduces the threshold for CDK activation (Figure 2D). These evidences support the view that G2/M transition in *O. tauri* is similar to the control network found in unicellular fission yeast, *Xenopus* egg extract and in mammalian cells. Although we could find evidence that support the similarity in terms of Cdk1 inhibitory phosphorylation it is still not clear whether CDK counteracting phosphatase is also regulated as observed in other organisms.

3.5. GWL, ENSA and/or ARPP19 might be found in *O. tauri* to control G2/M

Recently it has been shown that the regulation of mitotic entry is even more complex both in *Xenopus egg* extracts and mammalian cells. A new kinase called Greatwall (GWL) was identified which gets activated at G2/M parallel to the mitotic CDK/cyclin complex activation (Lorca & Castro, 2013). The active GWL is able to phosphorylate its substrates, called ARPP19 and Endosulphine (ENSA) respectively (Lorca & Castro, 2013). Both phosphorylated ARPP19 and ENSA bind and inhibit PP2A. This inhibition of CDK counteracting phosphatase results in the phosphorylation of substrates by mitotic CDK (Lorca & Castro, 2013). Since mitotic entry in *O. tauri* shows similarities to the G2/M transition found in *Xenopus egg* extracts and mammalian cells, we explored whether these genes (i.e. GWL, ARPP19, ENSA) might be present in *O tauri* genome.

First with sequence similarity searches two potential candidates were detected that might be coding for GWL in *O. tauri* (NCBI-Gene ID: 9831828 and 9837288). Human GWL has three isoforms and these putative genes show more than 30% homology to all the three human

isoforms (see Table 1). One of the candidate actually codes a Ser/Thr kinase (NCBI-Gene ID: 983182) in *O. tauri* and has 50% homology to the GWL found in *Arabidopsis thaliana*. Further, we found that this gene is present in *Chlamydomonas reinhardtii* with 99% similarity (Table 1).

Labandera et al. (2015) have recently identified both ENSA and ARPP19 in plants (Labandera et al., 2015). They also confirmed that GWL-phosphorylated key inhibitory sequence FDSADW was conserved across plants suggesting an ancient origin and conserved function of PP2A regulation (Labandera et al., 2015). Although ARPP19 and ENSA orthologues were found in more than twenty various organisms, even in *Chlamydomonas reinhardtii*, there is no evidence of these in *O. tauri* (Labandera et al., 2015). Our sequence similarity searches identified some potential ENSA and/or ARPP19 candidates in *O. tauri*, but the sequence of these genes did not show high similarities to the human ones. We observed query coverage of maximum 6 and 11% in these cases (Table 1). We also tried to find the similarity with the *Arabidopsis* or *Chlamydomonas* homologues of both ARPP19 and ENSA, but the maximum query coverage observed was only 17% (Table 1).

In the next step, we focused on identifying proteins that have GWL inhibitory sequence in *O. tauri*. Since this sequence is different in vertebrates (FDSGDY) and plants (FDSADW) we analysed both possibilities in *O. tauri*. We did not find any protein in *O. tauri* genome containing neither FDSGDY nor FDSADW sequences (Table 2). However many proteins were categorized based one or two amino acid difference in these phosphorylation motif of GWL. Checking the sequence similarity of these proteins to both ARPP19 and ENSA found in human, *A. thaliana* and *C. reinhardtii*, we found a relatively high sequence similarity of four potential candidates with human (see the NCBI-Gene IDs in red in Table 2).

Our analysis suggest that Greatwall/PP2A pathway might be present in the cell cycle of *O. tauri*.

4. Conclusions and future direction

The most well-known difficulties of cell cycle research are the complexity and redundancy of cell cycle genes in higher eukaryotes (Morgan, 2007). Therefore the cell cycle studies are focusing on using simpler eukaryotes as model organisms. Our study highlights that *O. tauri* (van Ooijen et al., 2012) can be used to explore the mechanism of cell cycle transitions in eukaryotes. *O. tauri* is one of the smallest free-living organisms. It has a minimal cellular organization with a single chloroplast and only one mitochondrion (Robbens et al., 2005). Recently, *O. tauri* has been successfully used to study the basic mechanisms of plant circadian clock due to its simple regulatory network of daily rhythm (Pfeuty et al., 2012, Thommen et al., 2012). Experimental studies in *O. tauri* provide pieces of evidence of different players involved in G1/S and G2/M transitions. We have developed mathematical models to integrate these experimental findings and also applied bioinformatics approaches to predict the other components and their connections that are relevant for G2/M transition.

By genome-wide analysis the core cell cycle genes in unicellular green algae have been identified (Robbens et al., 2005). Assuming regulatory connections between them based on studies in higher eukaryotes, we highlight the irreversible switch like characteristic of both G1/S and G2/M transitions in *O. tauri* (Figure 1 and 2). Since commitment seemed to be controlled by CDKA/E2F/Rb and mitotic entry is regulated by CDKB/Wee1/Cdc25 we suggest that *O. tauri* cell cycle is closely related to mammalian cell cycle. Most of the cell cycle studies are based on yeasts, however Rb pathway is missing from *Schizosaccaharomyces pombe*, while SBF/Whi5 regulation found in *Saccharomyces cerevisiae* show little sequence similarity with Rb network components even though they share similar topology. Although the regulatory connections between the key cell cycle controllers need to be further studied, *O. tauri* due to its simplicity might be a potential model organism to study eukaryotic cell cycle in the future.

We observed that the amino acid sequence of serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B (NCBI-Gene ID: 9834125) identified in *O. tauri*, shows 95% sequence similarity with its human orthologue (Table 1). This suggests that PP2A might be the CDK counteracting phosphatase in unicellular green algae. Further, we explored the possibility of Cdk counteracting phosphatase regulation involving GWL, ENSA and ARPP19. We identified two potential candidates for GWL and one of the genes (NCBI-Gene ID: 9831828) is highly related to GWL found in *Chlamydomonas reinhardtii* (amino acid sequence similarity is 99%) and shows almost 35% similarity to its human orthologue (Table 1), suggesting that the presence of GWL is evolutionarily conserved. The sequence similarity searches were not able to identify neither ENSA, nor ARPP19 homologues in *O. tauri* with great certainty. However, using well-known human or plant phosphorylation sites of GWL, we identified four potential candidates as GWL substrates, which have relatively high sequence similarity with human ENSA and/or ARPP19 (Table 2). Taking into consideration that length of potential candidates, the most possible one is NCBI-Gene ID: 9834557 in *O. tauri* since its length is very similar to the human ENSA and ARPP19. However the confirmation of presence of ENSA/ARPP19 proteins in *O. tauri* needs to be explored experimentally.

We have shown previously that antagonism between CDKB/Cyclin B and PP2A mediated via GWL and ENSA/ARPP19 (Figure 3A) contribute towards a switch like characteristic of the G2/M transitions (the original version of this mathematical model found in (Vinod & Novak, 2015)). Figure 3B shows the phase plane portrait describing the relationship between CDKA/CyclinB and ENSA for different total concentration of Cdc25 (Figure 3B). At very low concentration of Cdc25T both ENSA and CDKB/CyclinB are inactive (see filled black circle when Cdc25T=0.001 on Figure 4B) and the cells remain in G2. At high Cdc25T concentration (Cdc25T=1.5), the system has a stable steady state with high level of both

pENSA and CDKB/CyclinB suggesting the mitotic entry in *O. tauri*. At a certain range of Cdc25T level the balance curve has three interactions with two stable states and based to the initial conditions the cell can be either G2 or M phase (see green curves on Figure 4B). Our study highlight that G2/M transition in *O. tauri* might be mediated by multiple feedback loops that ensures robust switch like transition. Further, it is also possible that the mitotic entry regulation is much simpler in unicellular green algae than in higher eukaryotes and GWL and/or CDK might directly inhibit PP2A via phosphorylation. Out of 40 Ser and 22 Thr amino acids found in PP2A, we identified one Ser with the following FXSADX sequence (GWL phosphorylates Ser on FDSADW phosphorylation motif in plants) that can be phosphorylated by GWL (Table 3). We also found that PP2A can also be directly phosphorylated by CDK (Table 3).

In addition, the one copy of each putative orthologues of APC core elements (such as APC1, APC2 and APC3) and APC activators, i.e. CDH1 and Cdc20 have been also found in the *O. tauri* genome (Robbens et al., 2005). Therefore, similar to higher eukaryotes, we assume that CDKA/Cyclin A inhibits CDH1 by phosphorylation, meanwhile CDH1 directly acts on CDKA/Cyclin A by promoting the proteasome-dependent degradation of Cyclin A (Figure 4). Corresponding to already well-known regulatory connections from higher eukaryotes we suggest that the inactive state of CDKB/Cyclin B in G1 is guaranteed by a double negative feedback loop between CDKB/Cyclin B and CDH1 (Figure 1). If we consider that the Cyclin B level decreases at mitotic exit with the activation of APC/C^{Cdc20} and Cdc20 gets activated via CDKB/Cyclin B a negative feedback loop will be generated in the control network (Figure 4). This model would be able to generate a sustained oscillation of *O. tauri* cell cycle. Assuming that CDK counteracting phosphatase can dephosphorlyate Cdc20, the oscillatory characteristic of cell cycle machinery becomes even more robust. However our interesting hypotheses have to be explored by experiments.

The purpose of our research was originally to analyse the cell cycle machinery of *O. tauri* and demonstrate how the cell cycle regulation of this ancient species resembles the much more complex plant ones. With building up a mathematical model we planned to prove that these unicellular algae can be reliably used to study plant cell cycle. To our surprise, however, we found that *O. tauri* cell cycle control network is much more similar to mammalian cell cycle regulation. In this respect, it seemed to even prefer the yeasts, therefore we emphasize that *O. tauri* might be a useful model organism to understand the mammalian cell cycle. Our results also suggest that cell cycle regulation of mammals from ancient unicellular eukaryotes has basically remained unchanged; meanwhile a multicellular plant was much more differentiated. We suppose that the complexity of mammalian cell cycle control network ensures the robustness of the organism at various external and internal signals; however the irreversible one-way directionality of cell cycle transitions, with the same accuracy, is already guaranteed in the ancient unicellular eukaryotic cell.

Acknowledgment

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Competing Interests

The authors declare that they have no competing interests.

Table legends

gene of interest	*Ostreococcus t.* gene	query gene		sequence similarity
	NCBI-Gene ID	name	NCBI-Gene ID	
PP2A	9834125	PPP2R2D - human	55844	95%
	9834125	PPP2R2D - *Ara. t.*	5725732	98%
	9834125	PPP2R2D - *Chl. r.*	838348	95%
GWL	9831828	GWL - isoform I - human	84930	35%
	9831828	GWL - isoform II - human	84930	32%
	9831828	GWL - isoform III - human	84930	35%
	9837288	GWL - isoform I - human	84930	31%
	9837288	GWL - isoform II -human	84930	35%
	9837288	GWL - isoform III - human	84930	31%
	9831828	GWL - *Ara. t.*	821054	46%
	9837288	GWL - *Ar. t.*	821054	25%
	9831828	GWL - *Chl. r.*	5716867	99%
	9837288	GWL - *Chl. r.*	5716867	77%
ENSA	9832453	ENSA - human	2029	6%
	9831874	ENSA - *Ara. t.*	827304	15%
	9836702	ENSA - *Chl. r.*	5716065	17%
ARPP19	9832453	ARPP19 - human	10776	11%
	9834447	ARPP19 - *Ara. t.*	843284	12%
	9831167	ARPP19 - *Chl. r.*	5716863	10%

Table 1. Detecting the presence of PP2A, GWL, ENSA and ARPP19 homologues in *Ostreococcus tauri*. The putative PP2A, GWL, ENSA and ARPP19 homologues were identified and the percentage of sequence similarity was calculated by using Align Sequences Protein BLAST from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The genes of interest were checked in humans, *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*, respectively. Colour code of sequence similarity: red – 91-100%, orange – 25-90%, blue – 0-24%.

Ostreococcus t. gene NCBI-Gene ID	motif	sequence similarity human ENSA NCBI-Gene ID: 2029
none	FDSGDY	
none	XDSGDY	
none	FXSGDY	
none	FDSXDY	
9833269	FDSGXY	11%
none	FDSGDX	
9834557	XXSGDY	79%
9835513	XDSXDY	34%
9833269	XDSGXY	11%
9838599	XDSGDX	18%
9832493	FXSXDY	16%
9833501	FXSGXY	8%
9831982	FXSGDX	1%
9833269	FDSXXY	11%
9831843	FDSXDX	14%
9833832	FDSGXX	20%

Ostreococcus t. gene NCBI-Gene ID	motif	sequence similarity human ARPP19 NCBI-Gene ID: 10776
none	FDSGDY	
none	XDSGDY	
none	FXSGDY	
none	FDSXDY	
9833269	FDSGXY	9%
none	FDSGDX	
9834557	XXSGDY	75%
9835513	XDSXDY	35%
9833269	XDSGXY	9%
none	XDSGDX	
9832493	FXSXDY	11%
9833501	FXSGXY	5%
9831982	FXSGDX	16%
9833269	FDSXXY	9%
9831843	FDSXDX	16%
9833832	FDSGXX	25%

Ostreococcus t. gene NCBI-Gene ID	motif	sequence similarity *Chl. r.* ENSA NCBI-Gene ID: 5716065
none	FDSADW	
none	XDSADW	
none	FXSADW	
none	FDSXDW	
none	FDSAXW	
none	FDSADX	
none	XXSADW	
none	XDSXDW	
9838367	XDSAXW	5%
9834955	XDSADX	18%
none	FXSXDW	
9835631	FXSAXW	8%
9837702	FXSADX	20%
none	FDSXXW	
9831088	FDSXDX	30%
9838567	FDSAXX	13%

Ostreococcus gene NCBI-Gene ID	motif	sequence similarity *Chl. r.* ARPP19 NCBI-Gene ID: 5716863
none	FDSADW	
none	XDSADW	
none	FXSADW	
none	FDSXDW	
none	FDSAXW	
none	FDSADX	
none	XXSADW	
9835118	XDSXDW	9%
9835142	XDSAXW	17%
9834955	XDSADX	14%
9831650	FXSXDW	14%
none	FXSAXW	
9837702	FXSADX	4%
9831225	FDSXXW	4%
9831088	FDSXDX	4%
9838567	FDSAXX	13%

		sequence similarity
Ostreococcus t. gene	motif	*Ara. t.* ENSA
NCBI-Gene ID		NCBI-Gene ID: 827304
none	FDSADW	
none	XDSADW	
9837702	FXSADW	20%
none	FDSXDW	
none	FDSAXW	
none	FDSADX	
none	XXSADW	
9830818	XDSXDW	3%
9833522	XDSAXW	9%
9834955	XDSADX	18%
9837702	FXSXDW	20%
9837702	FXSAXW	20%
9837702	FXSADX	20%
none	FDSXXW	
9831088	FDSXDX	30%
9838567	FDSAXX	13%

		sequence similarity
Ostreococcus t. gene	motif	*Ara. t*. ARPP19
NCBI-Gene ID		NCBI-Gene ID: 843284
none	FDSADW	
none	XDSADW	
9837702	FXSADW	4%
none	FDSXDW	
none	FDSAXW	
none	FDSADX	
none	XXSADW	
9830818	XDSXDW	1%
9833522	XDSAXW	14%
9834955	XDSADX	14%
9837702	FXSXDW	4%
9837702	FXSAXW	4%
9837702	FXSADX	4%
9832567	FDSXXW	20%
9831088	FDSXDX	4%
9838567	FDSAXX	13%

Table 2. Detecting ENSA- and ARPP19-related genes in *Ostreococcus tauri*. The putative ENSA and/or ARPP19 candidate protiens were identified and the sequence similarity was calculated by using Align Sequences Protein BLAST from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The ENSA- and ARPP19-related proteins were searched according to their conserved phosphorylation sites by GWL. The proteins of interest were checked in humans, *Arabidposis thaliana* and *Chlamydomonas reinhardtii*, respectively. Corresponding to the test organism the given inhibitory sequences were used for the analysis (FDSGDY in vertebrates and FDSADW in plants). Colour code of sequence similarity: red – 91-100%, orange – 25-90%, blue – 0-24%.

putative role in *O. tauri*	NCBI-Gene ID	all potential P'ion sites		potential CDK P'ion sites (score>0.5)		potential GWL P'ion sites (score>0.5)	
		Ser	Thr	Ser	Thr	Ser	motif
PP2A	9834125	40	22	1	0	1	FXSADX
ENSA	9831088	42	20	7	1	1	FDSXDX
ENSA/ARPP19	9834557	8	7	0	0	0	XXSGDY
	9835513	28	12	3	1	1	XDSXDY
ARPP19	9833832	27	11	5	2	1	FDSGXX

Table 3. Bioinformatic analysis of CDK and GWL-dependent phosphorylation sites on PP2A in *Ostreococcus tauri*. The possible GWL and CDK phosphorylation site were identified by NetPhos 3.1. Ser and Thr phosphorylation sites having scores higher than 0.5 were collected.

Figure legends

Figure 1. G1/S transition is connected to circadian clock regulation in *Ostreococcus tauri*. (A) The simple wiring diagram of commitment point. Dashed lines represent how the molecules can influence each other. Blocked end lines denote inhibition. **(B) Signal response curve of G1/S transition in *O. tauri*.** The signal response curve of CDKA/Cyclin A is shown with the respect of Cyclin A synthesis (kscycA). Solid lines denote stable states, while dashed line denotes the unstable state. **Balance curves of G1/S transition (C) at physiological conditions, (D) when Cyclin A is over-expressed (kscycA'=1) or (E) Rb is down-regulated (RbT=0.1).** Balance curves for CDKA/Cyclin A (green) and Rb (red) are plotted. Along the balance curve the given component is maintained in steady state. Intersections of balance curves represent the stable (filled black circle) and unstable (open circle) steady states.

Figure 2. The mitotic entry regulation is Cdc25-dependent in *Ostreococcus tauri*. (A) The simple wiring diagram of G2/M transition. Dashed lines represent how the molecules can influence each other. Blocked end lines denote inhibition. **Balance curves of G2/M transition (B) at physiological conditions and (C) in HU block (Cdc25T=0.001).** Balance curves for CDKB/Cyclin B (green) and the total amount of CDKB/Cyclin B (red) are plotted. Along the balance curve the given component is maintained in steady state. Intersections of balance curves represent the stable (filled black circle) and unstable (open circle) steady states. **(B) Signal response curve of G2/M transition in *O. tauri*.** The signal response curve of CDKB/CyclinB is shown with the respect of Cdc25 level (Cdc25T). Solid lines denote stable states, while dashed line denotes the unstable state.

Figure 3. The hypothetic role of GWL and ENSA/ARPP19 in mitotic entry regulation in *Ostreococcus tauri*. (A) The simple wiring diagram of G2/M transition. Dashed lines represent how the molecules can influence each other. Blocked end lines denote inhibition. **(B) Balance curves of G2/M transition at physiological conditions.** Balance curves for CDKB/Cyclin B (green) and active ENSA (red) are plotted in an ENSA – CDKB/CyclinB coordinate system with various Cdc25T values. Along the balance curve the given component is maintained in steady state. Intersections of balance curves represent the stable (filled black circle) and unstable (open circle) steady states.

Figure 4. The hypothetic core network of *Ostreococcus tauri* cell cycle control network. Dashed lines represent how the molecules can influence each other. Blocked end lines denote inhibition.

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Appendix

1. The code for simulating signal response curves of G1/S transition

```
# a model to generate signal response curves of G1/S transition with XPP-AUT

# initial conditions to simulate G1/S transition
init CycA=0, kscycA'=0

# differential equations
# CycA represents the active CDKA/Cyclin A complex in the cell
dCycA/dt = kscycA*(E2FT - Complex) - kdcycA'*CycA

# kscycA represents the cAMP dependent CycA synthesis
dkscycA/dt = 0

# steady state functions
# Rb represents the active form of retinoblastoma (Rb) protein
Rb = RbT*GK(kdpRb,kpRb' + kpRb*CycA,JRb,JRb)

# E2F represents the active form of eukaryotic transcription factor
E2F = E2FT - Complex
BB = Rb + E2FT + (kdiss + kpRb' + kpRb*CycA)/kass
Complex = 2*Rb*E2FT/(BB + sqrt(BB^2 - 4*Rb*E2FT))
p RbT=1, kdpRb=0.5, kpRb'=0.1, kpRb=1, JRb=0.01
p kdcycA'=1, kdcycA=1
p E2FT=1, kde2f=0.01, JE2F=0.01, kpe2f=0.1, kpe2f'=1, kdiss=1, kass=1000

# 'Goldbeter-Koshand' function (GK)
GB(arg1,arg2,arg3,arg4) = arg2-arg1+arg2*arg3+arg1*arg4
GK(arg1,arg2,arg3,arg4) =
2*arg1*arg4/(GB(arg1,arg2,arg3,arg4)+sqrt(GB(arg1,arg2,arg3,arg4)^2-4*(arg2-
arg1)*arg1*arg4))

done
```

2.The code for simulating balance curves of G1/S transition

```
# a model to generate balance curves of G1/S transition with XPP-AUT

# initial conditions to simulate G1/S transition
init Rb=0, CycA=0

# differential equations
# Rb represents the active form of retinoblastoma (Rb) protein
dRb/dt = kdpRb*(RbT - Rb)/(JRb + RbT - Rb) - (kpRb' + kpRb*CycA)*Rb/(JRb + Rb)

# CycA represents the active CDKA/Cyclin A complex in the cell
dCycA/dt = kscycA*(E2FT - Complex) - kdcycA'*CycA
```

```
# steady state functions
# E2F represents the active form of eukaryotic transcription factor
E2F = E2FT - Complex
BB = Rb + E2FT + (kdiss + kpRb' + kpRb*CycA)/kass
Complex = 2*Rb*E2FT/(BB + sqrt(BB^2 - 4*Rb*E2FT))

# parameters
# simulating over-expression of CycA: kscycA=7.5
# simulating down-rgulation of Rb: RbT=0.1
p RbT=1, kdpRb=0.5, kpRb'=0.1, kpRb=1, JRb=0.01
p kscycA=1, kdcycA'=1, kdcycA=1
p E2FT=1, kde2f=0.01, JE2F=0.01, kpe2f=0.1, kpe2f'=1, kdiss=1, kass=1000

# 'Goldbeter-Koshand' function (GK)
GB(arg1,arg2,arg3,arg4) = arg2-arg1+arg2*arg3+arg1*arg4
GK(arg1,arg2,arg3,arg4) =
2*arg1*arg4/(GB(arg1,arg2,arg3,arg4)+sqrt(GB(arg1,arg2,arg3,arg4)^2-4*(arg2-
arg1)*arg1*arg4))

done
```

3. The code for simulating signal response curves of G2/M transition

```
# a model to generate signal response curves of G2/M transition with XPP-AUT
# initial conditions to simulate G2/M transition
init Mpf=0, Cdc25T=0

# differential equations
# Mpf represents the active CDKB/Cyclin B complex in the cell
dMpf/dt = kscycB - kwee*Mpf + k25*(CycT-Mpf) - kdcycB*Mpf

# Cdc25T represents the amount of in vitro Cdc25
dCdc25T/dt = 0

# steady state functions
# CycT represents the total amount of CDKB/Cyclin B complex in the cell
CycT = kscycB / kdcycB
# PP2 represents the active form of CDK counteracting phosphatase
PP2 = kapp2/(kapp2 + kipp2'*Mpf)
# wee represents the active form of Wee1 kinase
Wee = Vawee*PP2*Wee1T/(Vawee*PP2 + Viwee*Mpf)
# Cdc25 represents the active form of Cdc25 phosphatase
Cdc25 = (Va25*Mpf/(1+HU))*Cdc25T/(Va25*Mpf/(1+HU) + Vi25*PP2)
kwee   = kwee'*Wee1T + (kwee''-kwee')*Wee
k25    = k25'*Cdc25T + (k25''-k25')*Cdc25

# parameters
p kscycB=0.02, kdcycB=0.01
```

```
p kasi=100, kdsi=1
p kapp2=0.035, kipp2'=10
p kwee'=0.5, kwee''=30,Vawee=0.35,Viwee=0.5, Wee1T=1
p k25'=0.03, k25''=5, Vi25=0.35, Va25=0.5, Cdc25T=1, HU=0

done
```

4. The code for simulating balance curves of G2/M transition

```
# a model to generate balance curves of G2/M transition with XPP-AUT

# initial conditions to simulate G2/M transition
init Mpf=0, Cyct=0

# differential equations
# Mpf' represents the active CDKB/Cyclin B complex in the cell
dMpf/dt = kscycB - kwee*Mpf + k25*(CycT-Mpf) - kdcycB*Mpf

# CycT represents the total amount of CDKB/Cyclin B complex in the cell
dCycT/dt = kscycB - kdcycB*CycT

# steady state functions
# PP2 represents the active form of CDK counteracting phosphatase
PP2 = kapp2/(kapp2 + kipp2'*Mpf)
# wee represents the active form of Wee1 kinase
Wee = Vawee*PP2*Wee1T/(Vawee*PP2 + Viwee*Mpf)
# Cdc25 represents the active form of Cdc25 phosphatase
Cdc25 = (Va25*Mpf/(1+HU))*Cdc25T/(Va25*Mpf/(1+HU) + Vi25*PP2)
kwee   = kwee'*Wee1T + (kwee''-kwee')*Wee
k25    = k25'*Cdc25T + (k25''-k25')*Cdc25

# parameters
# simulating G2/M transition with various level of CycT: kscycB=0.001, 0.005, 0.01
# simulating HU block: HU=1000
p kscycB=0.01, kdcycB=0.01
p kasi=100, kdsi=1
p kapp2=0.035, kipp2'=10
p kwee'=0.5, kwee''=30,Vawee=0.35,Viwee=0.5, Wee1T=1
p k25'=0.03, k25''=5, Vi25=0.35, Va25=0.5, Cdc25T=1, HU=0

done
```

Contributions

O.K. and P.K.V. designed the study. O.K. and P.K.V. carried out the model simulations. O.K., P.K.V., B.N. and G.B. analysed and discussed the data. O.K., P.K.V., B.N. and G.B wrote the manuscript and gave the final approval for publication.

Figure 1

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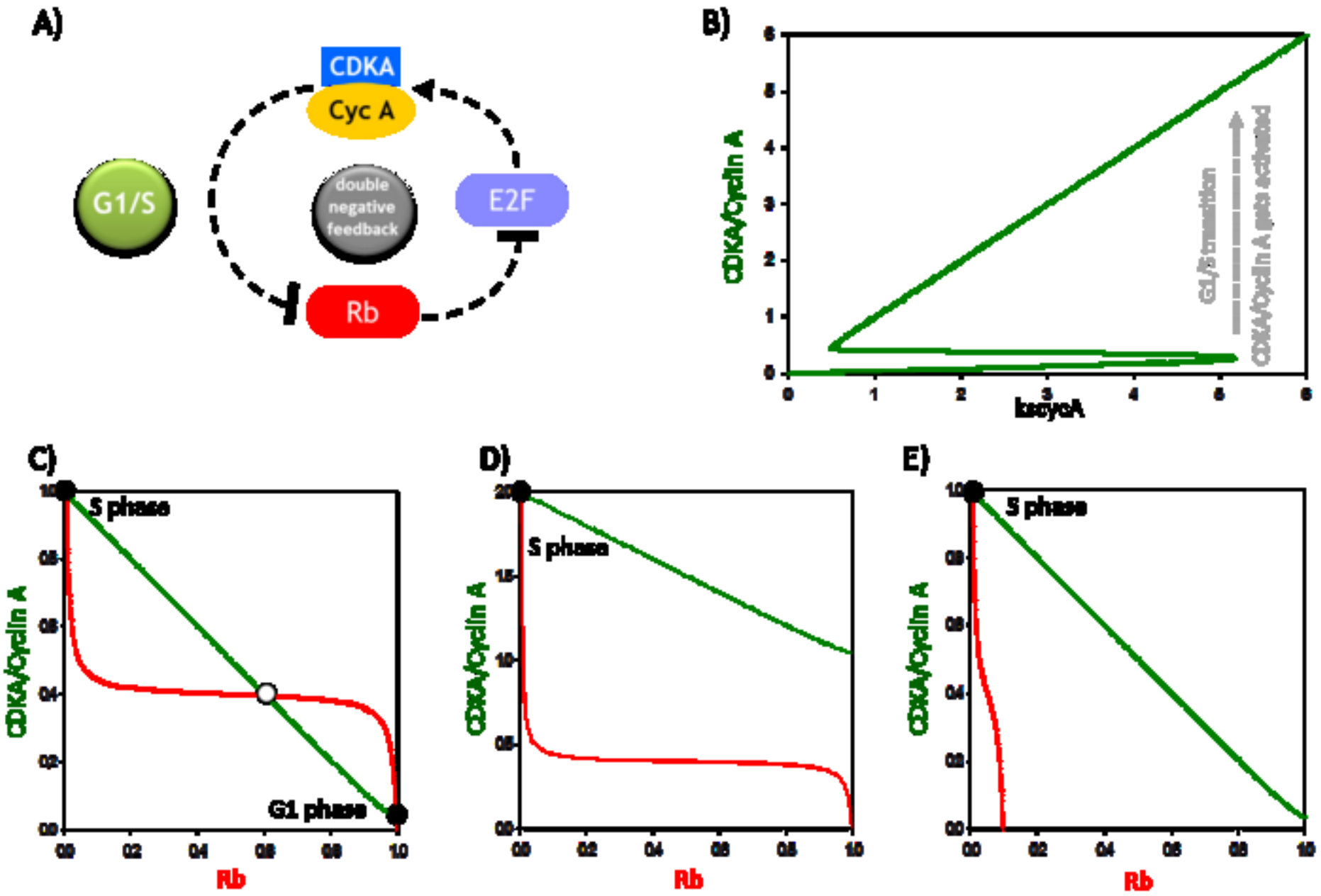

Figure 2

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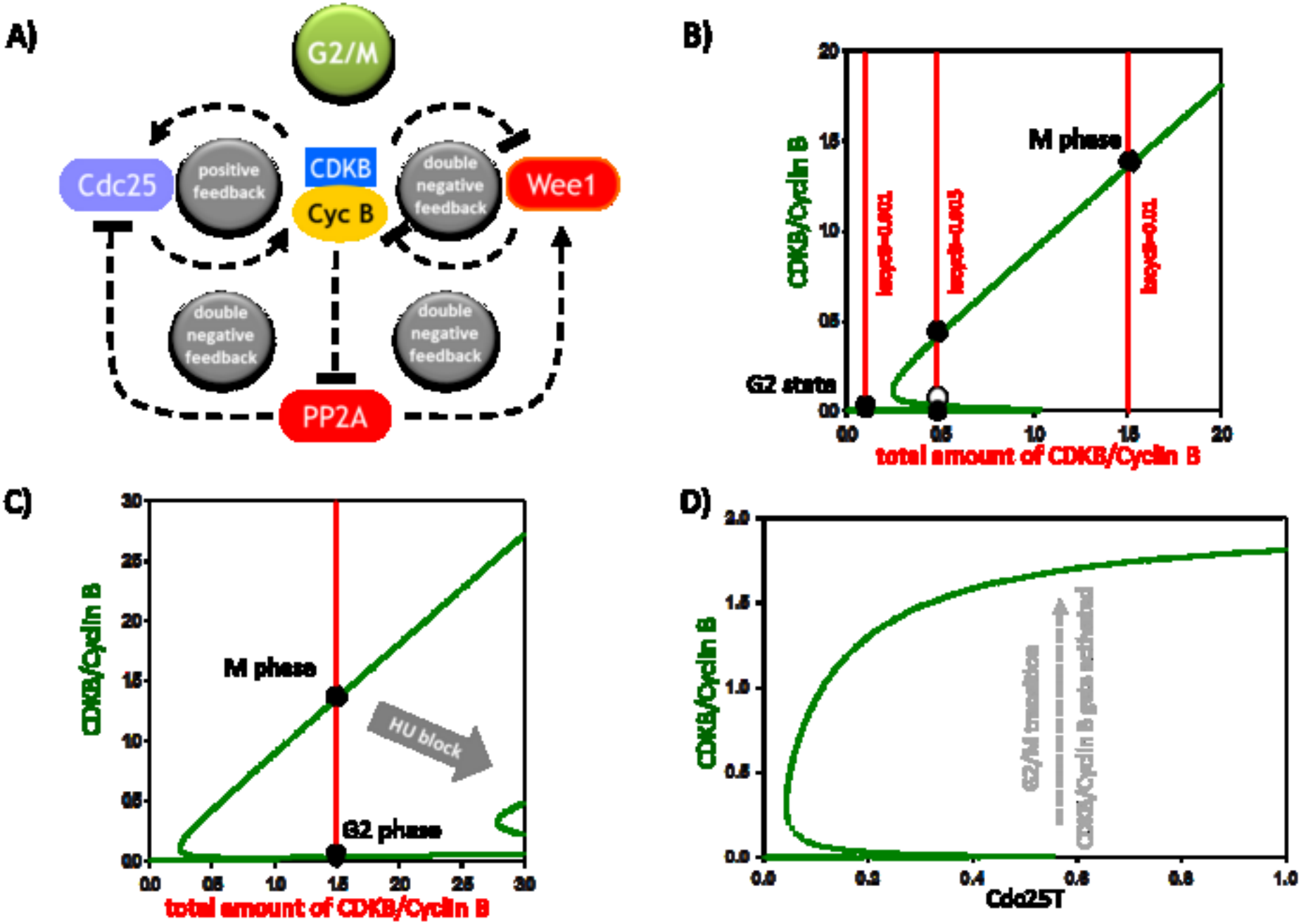

Figure 3

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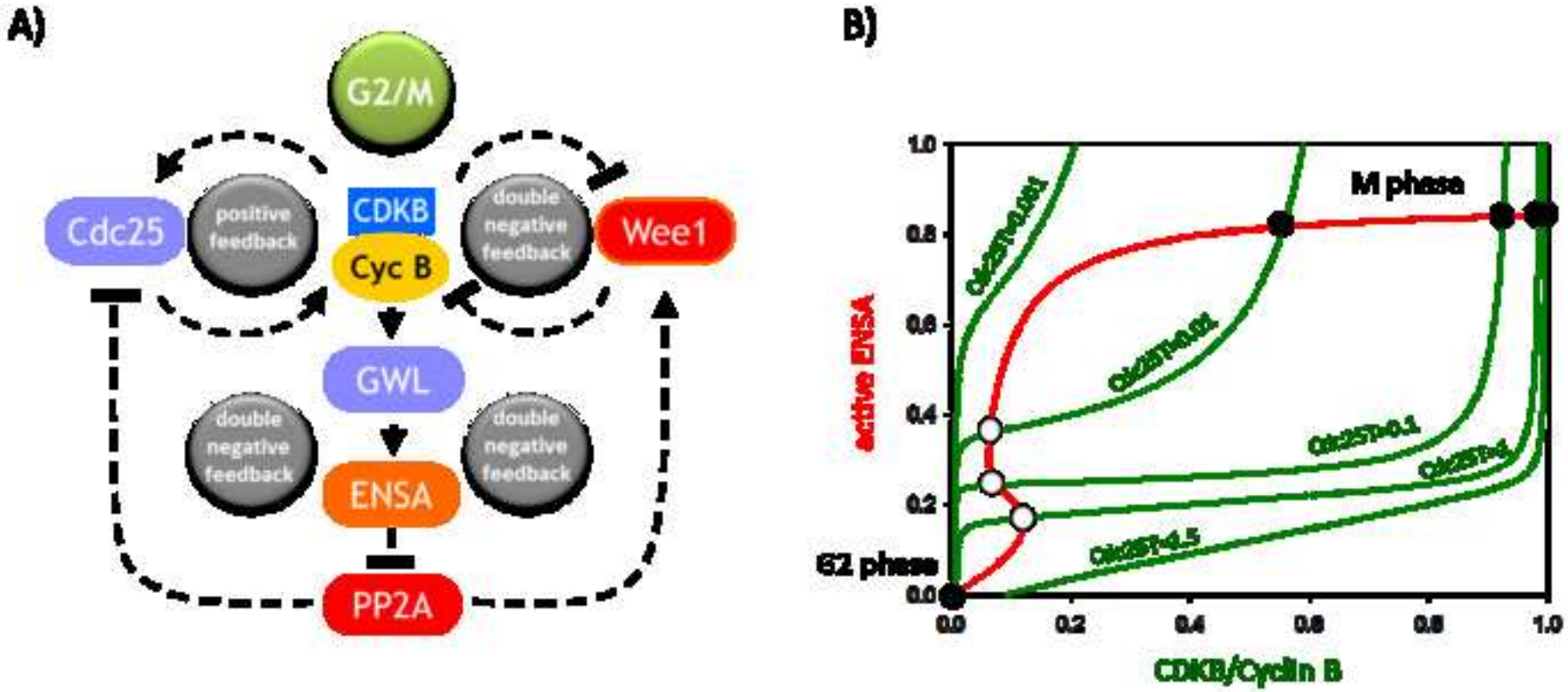

Figure 4

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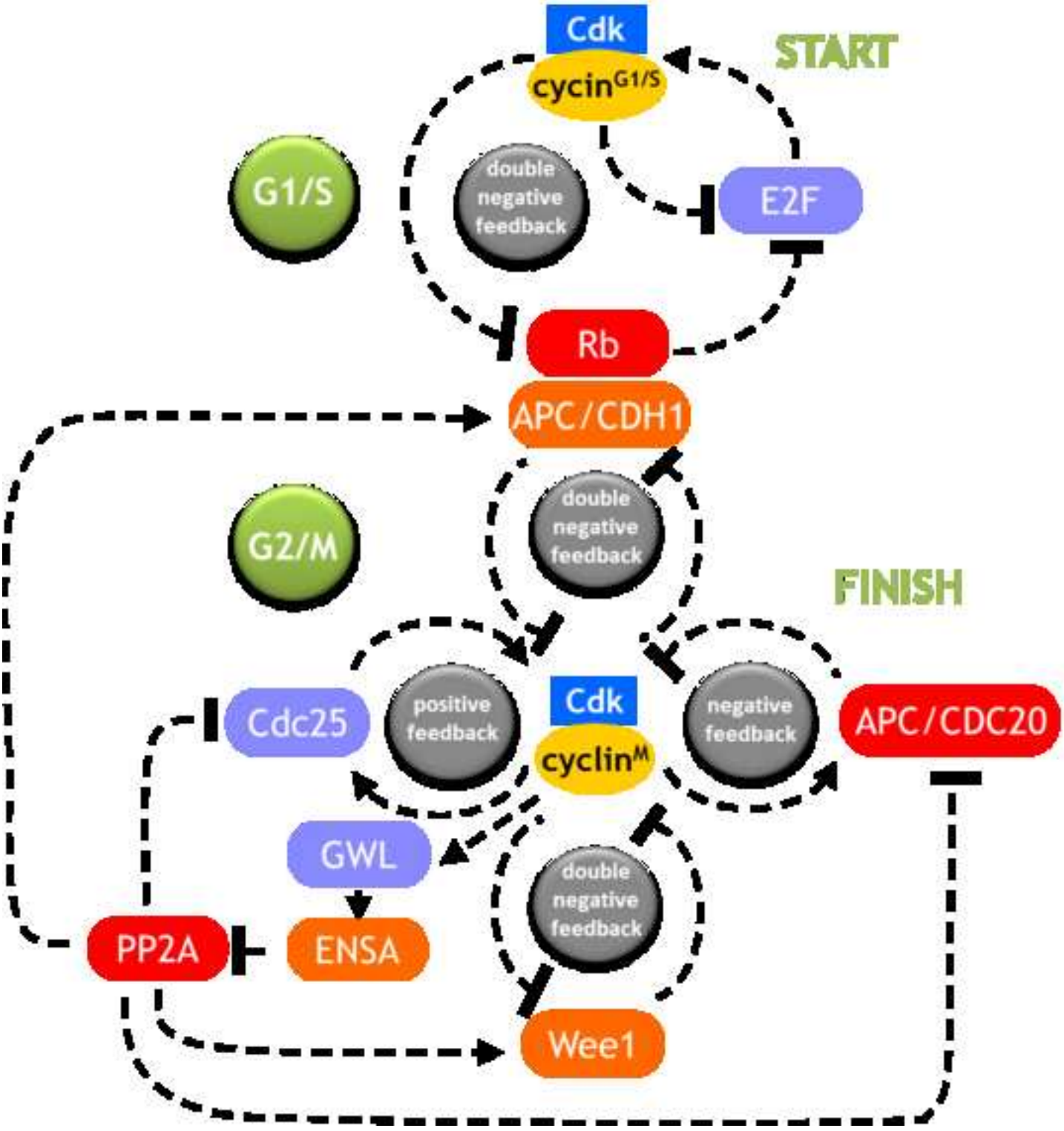

Supplementary material

Click here to download Supplementary material: Appendix_kapuy.docx