Text S2: Assumptions of the model

A common trend in developing complex models in molecular cell biology is to start from a simple model and then refine and expand it step by step (as more data become available) into an increasingly more comprehensive model. We have taken this approach in our study of mitotic exit in budding yeast cell cycle. We have limited the scope of our model so that it can be based largely on experimental observations, is not overwhelmed with assumptions, and is able to make predictions. Obviously, at any stage of modeling there will be facts that have not yet been incorporated and thus are out of the scope of the model. Major assumptions made in the process of building our model are listed below. Some technical assumptions of the mathematical model are described also in Supplementary Table S1.

1. We model average behavior of cells and do not address naturally occurring, stochastic fluctuations in cell cycle progression.
2. ODEs are given to describe the active forms (phosphorylated Cdc5, dephosphorylated Cdc15, Cdh1, and Net1) of the proteins for those who have both active and inactive form. The corresponding inactive form is given by: [total]-[active].
3. All synthesis, degradation, binding and dissociation reactions are described by mass action law. Rate constants starting with *k* have a dimension of min^{-1}, and other parameters are dimensionless.
4. Activation and inactivation of Tem1, reversible phosphorylation and dephosphorylation reactions of Cdc5, Cdh1, Net1, and Cdc15 are described by Michaelis-Menten (MM) kinetics.
5. Cdc28 and APC are neglected in the equations because they are constant. Clb2 represents Cd28/Clb2 complexes and Cdk represents the kinase activity of Cdc28-Clb2 dimers; in the absence of chemical inhibitor (INH) Clb2 represents the kinase activity. Similarly, Cdc20 and Cdh1 represent the concentration of active APC/Cdc20 and APC/Cdh1 complexes. Clb2 is synthesized at constant rate, and degraded by Cdh1 and Cdc20 [1-3].
6. The total amount of Cdc14 is observed to be constant through the cell cycle. Cdc14 contributes to Net1 dephosphorylation [4,5].
7. Cdc20 is synthesized at constant rate and degraded by Cdh1 [6-9].

8. Cdh1 is considered to be constant [7]. Cdc14 promotes Cdh1 activation by dephosphorylation, and Clb2 promotes its inactivation by phoshorylation [10-12]. Pds1 is synthesized at constant rate, and degraded by Cdc20 [13].
9. Esp1 is synthesized at constant rate and degraded with mass action kinetics. Esp1 forms complex with Pds1. In the complex, both Esp1 and Pds1 can be degraded.
10. Net1 is constant during the cell cycle. It is phosphorylated by Cdk/Clb2 [14], Polo kinase (Cdc5) [15,16], and active MEN (ACTMEN) [5,16-19], and dephosphorylated by Cdc14 and PP2A/Cdc55 [4,5]. We assume that Clb2, Cdc5, and ACTMEN phosphorylate Net1 both free and bound to Cdc14 in RENT, RENTP, and PRENT. Phosphorylated PRENT and PRENTP complexes dissociate quickly into PNet1, PNet1P, and Cdc14 whereas RENTP is a stable complex similar to RENT.
11. PP2A/Cdc55 is inhibited by Esp1 as (1+kpp*ki*Esp1)/(1+ki*Esp1).
12. S is a variable representing spindle elongation by the equation, S=exp((-ks)*(t-tm))*(1.0-*N*)*C*(1.0-*SCRRDD*). Parameter "*C*" represents cohesion cleavage. "*N*" is defined to simulate experiments with the drug nocadozole. In nocadozole, parameter *N* becomes 1, and consequently *S* becomes zero which means no spindle elongation. Similarly, *SCRRDD* is used to simulate "*GAL-SCC1-RRDD*" mutants. When cohesion is nondegradable, *SCRRDD* is set to 1.
13. Polo activity does not change quantitatively during mitosis [20]. Cdc5 is made at constant rate, and degraded by Cdh1 [21] with mass action kinetics. Cdc5 is activated by Cdk [22], and inactivated by a phosphatase with Michaelis-Menten kinetics.
14. "ACT" is used to represent the active form of the species. For example: in equation "ACTMEN=effc15*MEN", the parameter "effc15" quantifies the efficiency of the variable "MEN". When effc15 equals to zero, MEN becomes inactive, and when effc15 equals to 1, then MEN is fully active.
15. Tem1 concentration is constant. To simplify quantitative description, Tem1 is activated by Polo kinase and spindle elongation (represented by S), and inactivated by PP2A/Cdc55 and Cdc14 rather than acting through GAP and GEF. Tem1 has 3 forms, GDP bound form (TEM1i, inactive), GTP-bound free form (TEM1a), and a complex of Tem1-GTP and non-phosphorylated Cdc15, which is the active MEN.
16. MEN becomes inactive and falls apart if the Tem1 becomes Tem1-GDP (by PP2A and

Cdc14) or if the Cdc15 becomes phosphorylated (by Cdk). Cdc15 concentration is constant. Similarly, Cdc15 has 3 forms, non-phosphorylated Cdc15 (Cdc15a), phosphorylated (Cdc15P, inactive) and in the MEN complex. Cdk is the kinase that phosphorylates Cdc15a and makes it inactive, and Cdc14 is the opposing phosphatase.

17. We recognize the importance of spatial controls in the cell cycle. However, at this stage, we are trying to model the mitotic exit as far as possible without explicitly tracking the spatial localization of regulatory proteins. That would require a more sophisticated mathematical framework and is planned for the next stage of the model. Right after metaphase and before chromosome segregation, we keep track of proteins in the cell (in nucleus, cytoplasm, and SPBs). So, initial conditions of the variables are obtained for metaphase-arrested cells (*cdc20Δ GAL-CDC20*). Then they are released from the block by inducing expression of *CDC20* with galactose.

Since the consensus picture of the regulation of most ME proteins (summarized in Supplementary Text S1) has not changed significantly since the publication of Queralt's model, many of our modeling assumptions are the same as or similar to the assumptions made by Queralt et al. [23].

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