A comprehensive, mechanistically detailed, and executable model of the Cell Division Cycle in *Saccharomyces cerevisiae*

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ABSTRACT

Understanding how cellular functions emerge from the underlying molecular mechanisms is one of the principal challenges in biology. It is widely recognised that this will require computational methods, and we expect the predictive power of these models to increase as model coverage and precision of formulation increase. The most potent examples to date are the genome-scale metabolic models that revolutionised their field and paved the way for a bacterial whole-cell model. However, to realise the potential of whole-cell models for eukaryotes, we must enable genome-scale modelling of other cellular networks. This has proven particularly challenging for the cellular regulatory networks, i.e. signal transduction, gene regulation and cell cycle control. Here, we present a comprehensive mechanistic model of the molecular network that controls the cell division cycle in *Saccharomyces cerevisiae*. We use rxncon, the reaction-contingency language, to neutralise the scalability issues associated with regulatory networks, and to enable model formulation, visualisation and simulation at the genome-scale. In particular, we use a parameter-free simulation logic to validate the network and to predict genotype-to-phenotype relationships down to residue resolution. The genome-scale offers a new perspective on cell division cycle control: In contrast to conventional understanding, we find that the cell division cycle control network consists of disjunct control modules rather than a cycle, which synchronise three independent replication cycles. Taken together, we demonstrate that it is possible to build, visualise and simulate mechanistic models of cellular regulatory networks at the genome-scale, and that these models provide powerful tools to reconcile molecular biology and physiology - and to predict phenotypic effects of mutations down to the level of residue substitutions. We envisage these results as a key step towards eukaryotic whole-cell models and, in the long run, personalised medicine.

INTRODUCTION

Computational models provide powerful tools to study biological systems (Kitano 2002). In particular, mechanistic models that explain cellular functions and phenotypes from molecular events are powerful tools to assemble knowledge into understanding. When built carefully, they combine three functions: As integrated and internally consistent knowledge bases, as scaffolds for integration, analysis and interpretation of data, and as executable models. The value of these models arguably increases the more mechanistically detailed and comprehensive they are, culminating in the genome-scale mechanistic models of metabolism and the whole-cell model of *Mycoplasma genitalium* (Herrgard, Swainston et al. 2008, Karr, Sanghvi et al. 2012, Thiele, Swainston et al. 2013). These models can be used to explain and predict perturbation responses and genotype-to-phenotype relationships, and whole cell models have the potential to revolutionise biology, biotechnology and medicine. However, to realise this potential, we must be able to scale up modelling of all cellular processes.

This has proven especially challenging for the cellular networks that process information (reviewed in (Hlavacek and Faeder 2009, Münzner, Lubitz et al. 2017)). These networks encode information primarily through reversible state changes in their components, such as bonds or covalent modifications. Typically, a component can interact with multiple partners and be modified at multiple residues, and most of these bonds and/or modifications are not mutually exclusive. Hence, there is a one-to-many relationship between empirical observables (*elemental states*), such as a specific bond or the modification status at a specific residue, and the possible configurations of the protein (*microstates*). This leads to problems in most classical modelling formalism, where the resolution difference leads either to a loss of mechanistic detail (e.g. component level modelling) or to the combinatorial complexity (microstate modelling). The solution is to use a formalism with adaptive resolution, such as rule-based modelling languages (RBMLs (Blinov, Faeder et al. 2004, Danos, Feret et al. 2007)), the Entity Relationship diagrams of the Systems Biology Graphical Notation (SBGN-ER (Le Novere, Hucka et al. 2009)) or rxncon, the reaction-contingency language (Tiger, Krause et al. 2012) (reviewed in (Rother, Munzner et al. 2013, Chylek, Harris et al. 2015)). However, the potential of these formalisms has yet to be realised in a comprehensive mechanistic model of a eukaryotic signalling system.

The eukaryotic cell division cycle (CDC) may be the most interesting - both medically and philosophically - of these systems, as it constitutes the very core of life as we know it. The CDC is best understood in baker's yeast, *Saccharomyces cerevisiae*, where the rise and fall in activity of a single cyclin dependent kinase (CDK) suffices to drive the replication of DNA, nuclear division (including duplication and separation of the spindle pole body (SPB, the yeast centrosome)) and cell morphology and division (Enserink and Kolodner 2010, Howell and Lew 2012). The molecular basis of cell cycle control has been studied since the 1970-ies, when the first *cdc* mutants were identified (Hartwell, Culotti et al. 1970). It has also been a popular target of computational analysis, and has been studied with models ranging in complexity from conceptual oscillators via logical networks to extensive ODE systems that can predict most of the known mutant phenotypes (Goldbeter 1991, Chen, Csikasz-Nagy et al. 2000, Chasapi, Wachowicz et al. 2015, Kraikivski, Chen et al. 2015). However, even the largest of these models are limited in scope and cannot easily be extended to the genome scale. On the other hand, Kaizu and colleagues compiled the state-of-the-art knowledge in an exquisitely detailed map of the molecular network and its connections to a set of cellular signalling pathways (Kaizu, Ghosh et al. 2010), using the process description diagram language (SBGN-PD; (Le Novere, Hucka et al. 2009)). However, these maps are not executable and cannot be used for simulation. To realise the potential of a mechanistic

genome-scale model, we need to combine the features of all three efforts: The mechanistic precision in the molecular biology, the scope of the comprehensive maps, and the executability of the mathematical models.

Here, we present a mechanistic, executable and genome-scale model based on the network that controls and executes the cell division cycle in baker's yeast, *Saccharomyces cerevisiae*. We chose to build this model using rxncon, as it (similarly to RBMLs) has the adaptive resolution required to reconcile the necessary scalability and precision, and as rxncon networks (in contrast to rule-based models (RBMs)) can be compiled into and simulated as bipartite Boolean models (Romers, Thieme et al. 2017). This scalable, parameter-free simulation logic makes it possible to qualitatively simulate the cell cycle network, which is too large and/or has too many unknown parameter values (or truth tables) to be simulated with classical methods.

Based on an in-depth literature review, we compiled a mechanistically detailed model including 357 components and 790 elemental reactions of which 598 have regulatory contingencies. Despite the coverage, we could not verify that the regulatory network forms a closed cycle. Instead, it appears to consist of three disjunct control modules gating the G_1/S, G_2/M and M/G_1 transitions, which control and respond to the progression of three replication and division cycles: DNA replication, nuclear division and cell division (including bud emergence, growth, morphogenesis and cytokinesis). We added three coarse-grained "macroscopic cycles" and an interface to the control network, to close the regulatory gap through a hybrid model. The corresponding parameter-free model reproduces both starvation induced G_0 arrest and ordered progression through the three division cycles in the presence of nutrients, as well as arrest in the presence of pheromone (G_1-phase), hydroxyurea (HU, S-phase), latrunculin-A (LatA, G_2/M-phase) and nocodazole (NOC, G_2/M-phase). To test this model, we analysed a set of 85 mutants, including residue point mutations, and accurately predicted the viability or lethality of 72%. Finally, we visualised the complete regulatory structure in a rxncon regulatory graph, providing a poster-sized overview of the current mechanistic knowledge without any simplifying assumptions. Taken together, we show that it is possible to compile, visualise and analyse comprehensive mechanistic models of eukaryotic signal transduction, and that a qualitative network definition enables systems level predictions on genotype-to-phenotype relationships, down to residue resolution, without parametrisation or model optimisation. We envisage this as a stepping stone towards eukaryotic whole-cell models, and towards truly personalised medicine.

METHODS

The cell division cycle model was created using the second generation rxncon language (Romers and Krantz 2017), as described in the supplementary materials and elsewhere (Romers, Thieme et al. 2018). The rxncon model consists of two types of statements that both correspond directly to empirical data. First, *elemental reactions* define decontextualised reaction events in terms of changes in elemental states. An elemental reaction is essentially a reaction centre in a RBM (Faeder, Blinov et al. 2009). Second, *contingencies* define constraints on the elemental reactions in terms of (Boolean combinations of) elemental states. The complete set of contingencies for a single elemental reaction define the reaction context(s) in a RBM, but in rxncon the context definition is separated over several contingencies that each define the impact of a single elemental state (Romers and Krantz 2017). This gives a one-to-one correspondence between model definition and empirical data, improving composability as model changes and extension have a local impact only. Multiple contingencies can be combined into statements that are arbitrarily complex, defining reactions down to microstate resolution when necessary. Typically, however, single or few contingencies per elemental reactions suffice to capture all the empirically known regulatory mechanisms. In the end, the rxncon model constitutes a molecular biology knowledge base in formal language, which is computer readable, can be automatically visualised and compiled into a uniquely defined executable model.

Parameter-free models were created automatically using the rxncon compiler (Romers, Thieme et al. 2017), and simulated with the R package BoolNet (Mussel, Hopfensitz et al. 2010). The rxncon software can be downloaded from https://github.com/rxncon/rxncon or directly installed from the python package index ("pip install rxncon"). See https://rxncon.org for further instructions.

The cell division cycle model was visualised as rxncon regulatory graphs (Tiger, Krause et al. 2012), using Cytoscape (http://www.cytoscape.org/) and a visual formatting file (rxncon2cytoscape.xml; https://github.com/rxncon/tools).

The cell division cycle model is compiled in an SBtab compatible spreadsheet (Lubitz, Hahn et al. 2016), and is available from https://github.com/rxncon/models (CDC_S_cerevisiae.xls). The model is fully referenced through the "reference" columns in the reaction and contingency sheet, and explained in detail in the supplementary material.

The model is based on the literature listed in Table S3, as specified for individual reactions and contingencies in Table S1.

RESULTS

A comprehensive, mechanistic and executable model of a eukaryotic cell division cycle

We present a comprehensive, mechanistically detailed and executable model of the cell division cycle in baker's yeast (Figure 1A, Table S1, Figure S1 (printable A0)). The model includes regulated expression and degradation of proteins, assembly and regulation of the cyclin dependent protein kinases Cdc28 (Cdk1) and Pho85 (Cdk5), and the regulation of DNA replication, SPB duplication and nuclear division, and cell polarity and morphogenesis. Each of these processes is defined – as far as empirical knowledge allows – down to the role of specific modifications and bonds at particular residues and domains. The model accounts for 357 components. This number includes 229 individual proteins, and the genes and mRNAs of the 44 proteins for which we consider regulated expression and/or degradation. In addition, the model includes 7 multimeric protein complexes (e.g. APC/C), 3 chromosomal features (e.g. the origins of replication), 29 kinase and phosphatase activities as target specific enzymes that remain to be mapped on one or more gene products (e.g. Sfi1PPT as an unidentified phosphatase (PPT) of Sfi1), and one small molecule: phosphatidylinositol (PI). These components take part in 790 elemental reactions that produce and consume 1238 elemental states, and that are regulated by 598 contingencies - several of which correspond to Boolean combinations of elemental states.

In addition to the molecular reaction network (MRN), we encode a coarse-grained model (CGM) of DNA replication, SPB duplication and budding in 12 macroscopic reactions and 12 macroscopic states (Figure 1B). The macroscopic reactions respond to a series of inputs that correspond to outputs (observables) of the MRN. Conversely, the CGM provides outputs that act as inputs to the MRN. The entire regulatory logic is encoded in a single hybrid rxncon model, encompassing 802 reactions and 972 lines of contingencies. The contingencies connect the MRN and CGM to each other, and to the five external inputs we consider: Nutrients, Pheromone, HU, LatA and NOC. The biology and implementation is described and visualised in detail in the supplementary documentation. The model accounts for all components to which we could assign a mechanistic function in the control of cell cycle division, and hence it constitutes a first draft of a genome-scale mechanistic model of eukaryotic cell division.

Building a mechanistic knowledge base at the genome scale

The rxncon model constitutes a biological knowledge base rather than a mathematical model. We used an iterative workflow with information retrieval and interpretation, data formalisation, visual validation, computational validation and finally model analysis (Romers, Thieme et al. 2018). Through in-depth literature review, we extracted and evaluated empirical information and formalised it as reactions and contingencies using statements such as: "Cdc28 phosphorylates Sld2 at Threonine 84" (an *elemental reaction*) and "Cdc28 phosphorylates Sld2 at Threonine 84, only if Cdc28 is bound to Clb5 (or Clb6)" (a *contingency*). This type of mechanistic model puts high demands on data coverage and quality, which cannot always be met. Hence, the model contains gap-filling assumptions that are indicated in the model annotation (Table S1). In addition to these specific assumptions, we also made the general assumptions that reversible states that are produced are reversed (e.g. all phosphorylated residues are dephosphorylated) and that synthesised components (proteins, mRNAs) are degraded. This leads us to introduce a set of 28 undefined phosphatase activities to compensate for the fact that these reaction types are understudied (Tiger, Krause et al. 2012).

We make a few exceptions from these general assumptions when proteins are highly stable (e.g. Mcm2-Mcm7) or when modifications are reversed by degradation (e.g. Sic1 phosphorylation). Furthermore, several proteins in the network are regulated at the level of localisation, which cannot be properly expressed in rxncon. We work around this limitation by mapping the effect directly on the elemental states that are responsible for the localisation changes. For example, the transcription factors Ace2 and Swi5 are regulated at the level of nuclear localisation, which in turn is regulated by phosphorylation in the nuclear localisation signal (NLS). In the model, we make promoter recruitment directly dependent on NLS phosphorylation, bypassing the spatial description without compromising the regulatory logic. However, for most of the network we find biochemical (for reactions) or combinations of biochemical and genetic (for contingencies) data that can be formalised in the rxncon language using 14 elemental reaction types (Table S1), creating a mechanistically detailed knowledge base of the network that controls and executes the cell division cycle.

Insights at the genome scale: Three independent replication programs

The mechanistic genome-scale model presents a new perspective on the cell division cycle. The model summarises the current mechanistic knowledge on information transfer and hence connections in the model represent direct and functional connections *in vivo*. Consequently, the modules that emerge are not artefacts of model assumptions, but reflect known biological function. Conversely, the lack of connection implies that no (known) direct and/or functional connections exist. In particular, there is very little interaction between the three duplication cycles that execute DNA replication, SPB duplication and nuclear division (ND), and budding, morphology and cytokinesis (CD). These processes do not directly interact at the molecular level outside mitosis. Instead, these processes are monitored and synchronised by the regulatory network. While the lack of direct mechanistic connections between the cycles may reflect missing knowledge, the current model predicts that the cell division cycle consists of three distinct programs that can be executed independently: DNA replication, nuclear division (including SPB duplication) and cell division. With the appropriate adaptation of (the state of) the regulatory network, it should be possible to execute these programs separately and hence uncouple DNA replication, nuclear division and cell division. Such uncoupling could generate ploidy shifts, multinucleate cells, or cells without nuclei; which have all been observed in eukaryotic cells. The perhaps most prominent example of the modularity of these processes is meiosis, where a single DNA replication (pre-meiotic S-phase) is followed by two rounds of nuclear division (meiosis I + II) without any cell division (Neiman 2011). Hence, the CDC control network gates the cell division cycle in response to cellular and/or environmental status to synchronise three independent cycles; DNA replication, nuclear division and cell division, during a normal cell division cycle.

Mechanistic modularity: The CDC control network is not a cycle but three major checkpoints

We made a number of striking observations in the model building process. While we only used previously published data, the holistic picture of the assembled knowledge highlighted features that were not obvious from the modules themselves. In particular, the regulatory network controlling the cell division cycle does not in itself form a cycle. Instead, the regulatory network falls apart into three modules, corresponding to three major checkpoints that gate the G_1/S, the G_2/M and the M/G_1 transitions (we found no evidence for a regulatory S/G_2 boundary). Judged on the available data, these three subnetworks only interact indirectly

through the progression of the DNA, ND, and CD cycles (Figure 1C). Arguably, this makes perfect sense, as the control network must be responsive to the state of the cell division cycle. At the same time, this is not widely recognised in the literature, and several current models of the cell division cycle directly link the three regulatory modules into a clock-like network. Our findings here suggest that this is incorrect.

Merging perspectives: A role for the DNA damage response in normal CDC control

The model also connects knowledge from several distinct areas of research. When we combined knowledge from the cell cycle and DNA damage research fields, a clear picture of how DNA replication is monitored and how this prevents mitotic entry emerged. Ongoing DNA replication leads to ssDNA/Rad53 signalling from replication forks (Figure 2). This signal maintains S-phase transcription (through inhibition of the Nrm1 repressor), and inhibits the G2/M transition through transcriptional inhibition of the *CLB2* cluster (through inhibition of the Ndd1 activator and through post-translational inhibition of Cdc28-Clb2 through stabilisation of the inhibitory CDK tyrosine kinase Swe1. While the S-phase checkpoint is well known from studies on DNA damage and repair, their function are again not (widely) recognised in the literature on the cell division cycle control. However, the essential function of Mec1/Rad53 signalling in normal cell cycle control suggests that this may be its primary function.

Executing the logic: Ordered progression of the cell division cycle and nutrient dependent G_0 arrest

We analysed the cell division cycle model through parameter-free simulation. The rxncon network is not directly executable, but compilable into a uniquely defined bipartite Boolean model (bBM; (Romers, Thieme et al. 2017)). We used the bBM to evaluate the completeness of the cell division cycle model in three steps: First, we searched for a point attractor in the absence of nutrients, reflecting G_0 arrest. Second, starting from this G_0 attractor, we released the cell cycle arrest through addition of nutrients and searched for a cyclic attractor with ordered progression through the three macroscopic cycles. Third, we interrupted the cyclic attractor by adding compounds known to halt cell cycle, i.e. Pheromones, HU, LatA or NOC. The first step was to find an appropriate initial state vector for the model. The model has 1403 nodes and hence 2^{1403} ($\sim 10^{422}$) possible state vectors, precluding an exhaustive search. Instead, we used the rxncon default vector where all neutral elemental states and state-less components are True and all other states, all reactions and inputs are False (see (Romers, Thieme et al. 2017)), and released it in the absence of nutrients (setting the placeholder [Histones] input to True). Interestingly, the resulting point attractor is consistent with nutrient dependent G_0 arrest (Fig 3A; Fig S2). Next, we simulated the network from this initial state in the presence of nutrients, and evaluated the simulation trajectory and attractor to identify inconsistencies and gaps in the knowledge base. Most importantly, the crude time concept in the Boolean model caused significant problems, as shorter event chains are faster even if they occur through slower reactions. To resolve this problem, we introduced a time-scale separation that made all transcriptional reactions and DNA replication slower than other reactions (such as post-translational modifications or protein-protein interactions), by requiring their input to be true for twenty consecutive time steps before firing (reflecting the longest signalling path). After introducing the time scale separation, the model accurately reflects the ordered progression through the three macroscopic cycles (Fig 3B; Fig S3), resulting in a cyclic attractor with

period 186. Finally, we examined the arrest point in response to Pheromone, HU, LatA and NOC (Fig 3C; Fig S4), identifying and correcting one final issue in the network: The ability of Swe1 to inhibit mitotic entry in the absence of a proper bud. The changes made in the network validation phase are summarised in Table 1.

Testing the model: Parameter-free simulation accurately predicts genotype-to-phenotype relationship for 63 out of 85 tested mutants

To examine the predictive power of the model, we analysed the predicted arrest point of *cdc* mutants with known phenotypes. We chose 85 (combinations of) deletions, point, and constitutive (over) expression mutants, and examined their cell cycle progression (cyclic attractor) or arrest (point attractor) (Table S2). We observe two types of cyclic attractors; a normal and ordered progression through all three macroscopic cycles (viability) and a partial cyclic attractor passing through DNA replication and nuclear division, but not cell division (resulting in multinucleate cells, scored as lethality). In addition, we observe twelve distinct point attractors (at the level of macroscopic states), which correspond to G_1, S, G_2/M, M, and T arrest (Fig 4). The model correctly predicts all 43 lethal mutants, and 19 out of 42 viable mutants, but also scores 23 viable mutants as inviable. We note that the model is conservative in estimating viability, and look closer at the 23 inconsistent predictions (Table 2). Several of these mutants can be explained by implicit synthetic lethality[1]. I.e., the mutations are synthetically lethal *in vivo* with components for which key mechanisms are not known and hence not included in the model. E.g., almost no mechanistic data exist on the function of Clb3 and Clb4, which can partially compensate for loss of Clb5, explaining why *clb5* mutants are predicted to be lethal in the model (they correspond to the lethal *clb3clb4clb5* triple mutants (Schwob and Nasmyth 1993)). Similarly, no strictly required mechanism for Bck2 is implemented, explaining the lethality of the *cln3* (*bck2cln3* (Epstein and Cross 1994)) mutant. Seven of the 23 inconsistent predictions can be explained through implicit synthetic lethality. Some of the remaining mutants can be explained by strong phenotypes, which could be justified as “lethal” on a binary scale, such as the SBF-mutants (*swi4*, *swi6*, *swi6S4A* and *swi4swi6*) and *pds1*. Others, like *mbp1* and *cdh1*, which are predicted to be lethal but have no or weak reported phenotypes, likely indicate missing redundancy in the model. Taken together, the biological knowledge base uniquely defines a parameter-free model, which accurately predicts the vast majority of the mutant phenotypes that we tested - including point mutants.

[1] Synthetic lethality: The combination of two or more mutations that are individually viable results in lethality.

DISCUSSION

We present a genome-scale, mechanistic model of the cell division cycle. It is mechanistic, as the connections within the model correspond to direct biochemical reactions or dependencies of these reactions on the state of the reactants, down to the level of specific residues and domains. It is genome-scale, as it accounts for all components in the cell division cycle for which we could assign a mechanistic function. We visualise the complete knowledge base as a single, connected network, and show that it defines an executable model. We use this model for validation of the network and to predict genotype-to-phenotype relationships down to the resolution of specific residues.

The genome-scale scope allows us to interpret missing features. The most striking observation is the lack of a single cycle: The control network falls apart into three distinct control circuits; G_1/S, G_2/M and M/G_1, which monitor and control three distinct replication cycles: DNA replication, nuclear division and cell division. While we cannot rule out as yet unknown connections between the three control modules and between the three replication cycles, the modularity we observe seems to make perfect sense: The control network needs to be more than a sizer/timer; it must also depend on the replication cycles to be able to control these processes. On the other hand, the three replication cycles need to be distinct in order to explain ploidy shifts, multi-nucleate (or nuclei-free) cells and meiosis. Nevertheless, the regulatory mechanisms that uncouple these cycles remain largely unknown even in baker's yeast.

These findings have implications for how we model the CDC. We and others have previously modelled the regulatory network as a closed cycle, where CDC progression may be nutrient dependent, but not explicitly dependent on the progression of the internal division events. In particular, it has been difficult to find a direct link from the G_1/S transition (Cln1/2, Clb5/6 expression) to mitotic entry (Clb1/2 expression). We and others have modelled this as a gene expression cascade (Clb5/6 -> Clb3/4 -> Clb1/2), but without being able to explain how this cascade responds to e.g. HU-induced S-phase arrest (Linke, Chasapi et al. 2017, Spiesser, Uschner et al. Submitted) . Here, we can explain this by linking DNA damage signalling to cell cycle progression, through inhibition of Clb1/2 at two distinct levels: Transcriptionally through inhibition of Ndd1, and post-translationally through the stabilisation of Swe1. This knowledge was available in the literature, as was the knowledge that *mec1* and *rad53* mutants are impaired in normal CDC-progression, but has previously not been brought together in cell cycle models. Based on the observations we make here, it seems that the interplay between the regulatory network and division events are a necessary component of a mechanistic cell cycle model.

Despite its scope, the model we present here comes with several limitations. First of all, it is primarily a biological knowledge base. We have focussed on the qualitative information, i.e., which state variables change, which reactions are responsible for these changes, and in which molecular context these reactions occur, as this information is more abundant and less uncertain - as it is for metabolic networks (Herrgard, Swainston et al. 2008). Second, the model is limited by literature data coverage and quality. While the CDC of *S. cerevisiae* is exceptionally well known, we nevertheless observe uncertainty and gaps in this knowledge. Here, we make a minimal set of assumptions to make the model functional, and consider these gap-filling additions hypotheses for future experimental validation. However, the mutant analysis also suggests that we are missing redundancies in the network, or that some effects cannot be properly described at the qualitative level only. The latter is particularly true for the macroscopic reactions we introduce to cover DNA replication, spindle pole duplication and

cell growth and cytokinesis. The mono- and bimolecular formulation (in the context of arbitrarily large complexes) is a useful abstraction level to describe signal transduction, but not to describe large cellular structures, polymers or quantitative properties of molecule pools (such as polarity establishment). The macroscopic placeholder reactions of the CGM must be turned into models of their own before we reach a quantitative predictive model of the cell division cycle. Third, the model does not explicitly include spatial aspects, as complex level properties (such as localisation) cannot currently be expressed in the rxncon language. Instead, we directly connect the states that regulate localisation to the reactions that depend on it. Fourth and finally, the Boolean modelling logic is a very crude approximation both quantitatively and temporally, and, indeed, we needed to introduce time-scale separation between transcription and the other signalling events. However, a quantitative simulation approach would require parameters, creating a massively underdetermined parameter-estimation problem. All things considered, we believe the parameter-free simulation - which reflects the qualitative model only - may be more informative, and, indeed, find that the vast majority of the mutant phenotypes (which were not used to validate or improve the model) can be reproduced even by this qualitative simulation method. Interestingly, the discrepancies between model predictions and known phenotypes seem to primarily identify missing redundancies, and hence missing empirical knowledge. Taken together, we present a first draft model, to be refined and extended by the community, which highlights the need for further dedicated work on the experimental characterisation of this fundamental model system.

This work is complementary to quantitative modelling efforts. The qualitative simulation we use here can indeed give a readout for 1403 variables - elemental reactions and states - but the values of these variables are limited to True or False. In addition, we project all this on a binary phenotype: Dead or Alive. In contrast, quantitative models can help us precisely predict quantitative or complex phenotypes, such as cell cycle duration, cell size distribution, and polarity establishment (Chen, Csikasz-Nagy et al. 2000, Giese, Eigel et al. 2015, Kraikivski, Chen et al. 2015). However, the model we present here is much larger in scope than these quantitative models. Until today, only the Kaizu map is similar in scope: This network contains 318 proteins, including 96 signalling components, leaving a cell cycle module of 230 proteins - of which 148 are represented in the model we present here (individually or in one of the protein complexes) (Kaizu, Ghosh et al. 2010). However, the Kaizu map is not executable and can hence be neither computationally validated, nor used for genotype-to-phenotype predictions. In the future, we may be able to reconcile the two: Scope and quantitative modelling. The model here could in principle be converted into a quantitative model, as a rxncon model is semantically defined in terms of a rule-based model in the BioNetGen language (Romers, Thieme et al. 2018). However, to simulate this network as a quantitative model, we need to overcome a number of hurdles: First, there is no clear equivalence to rxncon inputs in a rule based model, so the connections between modules must be hand crafted. Second, we would need to find or estimate values for >790 unique parameters. Third, we would need representations of the DNA replication, nuclear division, and cell division cycles that are compatible with agent based simulation. In the foreseeable future, it seems likely that meaningful simulation of signal transduction networks at this scale will remain qualitative or semi-quantitative, as it does for metabolic networks.

However, we show that we can build and visualise models of information processing networks that are mechanistically detailed, large scale and executable. Until today, this has only been done for metabolic (or mass-transfer) networks, and the mass-transfer logic leads to crippling scalability issues when applied to signal transduction networks (due to microstate enumeration; reviewed in: (Hlavacek and Faeder 2009, Chylek, Harris et al. 2015, Münzner, Lubitz et al. 2017)). This technical issue can be solved by using a formalism with adaptive

resolution, such as a rule-based modelling language (see e.g.:(Blinov, Faeder et al. 2004, Danos, Feret et al. 2007, Chylek, Harris et al. 2015)), SBGN-ER (Le Novere, Hucka et al. 2009) or rxncon (Tiger, Krause et al. 2012). We settled for rxncon, as it is text based rather than graphical, uses a higher-level biological language rather than model code, supports scalable visualisation, and is compilable into a parameter-free model for direct simulation (Romers and Krantz 2017, Romers, Thieme et al. 2017). In particular, we find the iterative visualisation in the regulatory graph invaluable for the model construction process, and we present the final graph in a wall-chart that is inspired by the biochemical pathway maps (Michal 2014). In addition, the ability to go from a pure biological knowledge base to a parameter-free model that can predict system level features from molecular mechanisms is highly non-trivial (Hlavacek and Faeder 2009). Here, we use this feature for network validation - by iteratively analysing and improving the network until the corresponding model reproduces wild-type behaviour - and for genotype-to-phenotype predictions of 85 mutants. Hence, we bring together the mechanistic precision in the molecular biology, the scope of the comprehensive maps, and the executability of the mathematical models.

Taken together, we show that it is possible to build, visualise and simulate mechanistic genome-scale models of eukaryotic signal transduction networks. Here, we do this for the cell cycle network of baker’s yeast, and, although the rxncon language must be extended to deal with e.g. alternative splicing and localisation, there are no fundamental differences to higher eukaryotes. A similar high-resolution knowledge base on the information processing network in human cells would be an important step towards a human whole-cell model and, when it accounts for the molecular effect of allele differences and drug perturbations, truly personalised medicine.

FIGURE LEGENDS

Figure 1: The molecular architecture of the CDC control network. (A) The model consists of seven parts: (i) Gene expression, (ii) regulated degradation, (iii) CDK assembly and activation, (iv) DNA replication, (v) SPB duplication and nuclear division, (vi) cell division, and (vii) a set of reactions involving cell cycle components but without (known) regulatory connections to the main network. The outputs of the mechanistic reaction network (MRN) in modules (iv)-(vi) determine the progression of the coarse-grained model (CGM). **(B)** The CGM consists of three parts: (left) SPB duplication and nuclear division, including satellite formation (SPB: neutral (0) -> satellite), duplication plaque formation (SPB: satellite -> duplication plaque), SPB duplication (SPB: duplication plaque -> SPB duplicated), SPB separation (SPB: duplicated -> bipolar), and nuclear division (SPB: bipolar -> neutral). (middle) DNA replication, including licencing (DNA: neutral (0) -> licensed), initiation (DNA: licensed -> replicating), replication (DNA: replicating -> replicated) and separation (DNA: replicated -> neutral). (right) Cell division, including: Bud emergence (Bud: none (0) -> small), bud growth (Bud: small -> large), and cytokinesis (Bud: large -> 0). The MRN and CGM parts communicate via an input/output layer. (C) Schematic representation of the hybrid model architecture. The three independent replication cycles are regulated by three disjunct control modules gating the G_1/S, G_2/M and M/G_1 transitions. The network is responsive to different forms of stress and other external cues, but here we only consider nutrients and four classical CDC arrest drugs: Pheromone, HU, LatA and NOC.

Figure 2: The Mec1/Rad53 pathway senses ongoing DNA replication to inhibit mitotic entry. Ongoing DNA replication leads to exposed ssDNA and ssDNA/dsDNA junctions at the replication forks, which are sensed by the Mec1/Rad53 pathway. Mec1 phosphorylates and stabilises Swe1, a Cdk1-Clb1/2 inhibitor, and Rad53 phosphorylates and inhibits Nrm1, which is needed for *CLB1/2* transcription. The regulatory graph is a bipartite representation where elemental reactions (red) produce or consume elemental states (blue), and elemental states influence - through contingencies - elemental reactions. Complex contingencies are collected in Boolean nodes (AND/OR/NOT; light grey), and Input/Output nodes (dark grey) define the interface between the model and its surroundings - and between the MRN and CGM parts.

Figure 3: The parameter-free model reproduces both cell cycle progression and arrest. Simulation results visualised on the CGM states only. (A) When the model is simulated with nutrients set to False, the cells arrest at G_0: With licensed DNA, SPB-satellite and no bud. (B) When cells are released from the G_0 arrest by setting nutrients to True, they progress through the cell division cycle by replicating their DNA, undergoing nuclear division and rapidly thereafter cell division, and resetting the network for a new division, traversing 186 Boolean steps. (C) The cells arrest again when exposed to pheromone (G_1: licensed DNA, SPB-satellite, no bud), HU (S: replicating DNA, duplicated SPB, large bud), LatA (G_2/M: replicated DNA, duplicated SPB, small bud) and NOC (G_2/M: replicated DNA, duplicated SPB, large bud).

Figure 4: The model predicts the phenotypes of a majority of all tested mutants. Simulations were performed by introducing deletions (setting all protein states (and mRNA and Gene, if appropriate) to False), point mutations (by locking proteins in a certain

modification state) or constitutive overexpression (by setting the transcription reaction to True constantly), and the mutants were analysed through simulation by release from the (modified) G_0 state of the wild-type. (A) The model predicts the correct phenotype (dead or alive) for 62 out of 85 mutants, although closer inspection revealed that several of the 23 inconsistent mutants can be explained by implicit synthetic lethality or strong phenotypes (Table 2). (B) The 62 mutants that are predicted to be dead arrest at thirteen distinct arrest points, that correspond to G_1 (1a-c, 5*, 6*), S (2), G_2/M (3a-e), M (4) and T (5*, 6*) arrest, or progress through a partial cyclic attractor with repeated DNA replication and nuclear division but no cell division, resulting in multinucleate cells. Note that group 5 and 6 contains G1 and T arrested cells that completed nuclear division but arrest before cytokinesis. They can only be distinguished on the DNA/Nuclei count (Table S3).

Table 1: List of gap-filling additions.

Table 2: List of inconsistent mutants. The model predicted 23 viable mutants to be lethal. Of these, seven mutants can be explained by implicit synthetic lethality: While *cln3* and *clb5* are viable, the model lacks implementation of mechanistic functions of Bck2 and Clb3/Clb4, which are necessary in the absence of Cln3 and Clb5, respectively. While other mutants have strong phenotypes in e.g. cell size or growth rate, such as *swi4*, *swi6* and *pds1*, others - like *cdh1* - have no strong phenotypes as single mutations, suggesting that redundancy mechanisms are missing in our current knowledge.

Fig S1: The molecular architecture of the cell division cycle. Available at https://github.com/rxncon/models/ as CDC_S_cerevisiae_poster.pdf.

Table S1: The rxncon model. Available at https://github.com/rxncon/models/ as CDC_S_cerevisiae.xls.

Table S2: List of references.

Table S3: List of mutants examined in this study.

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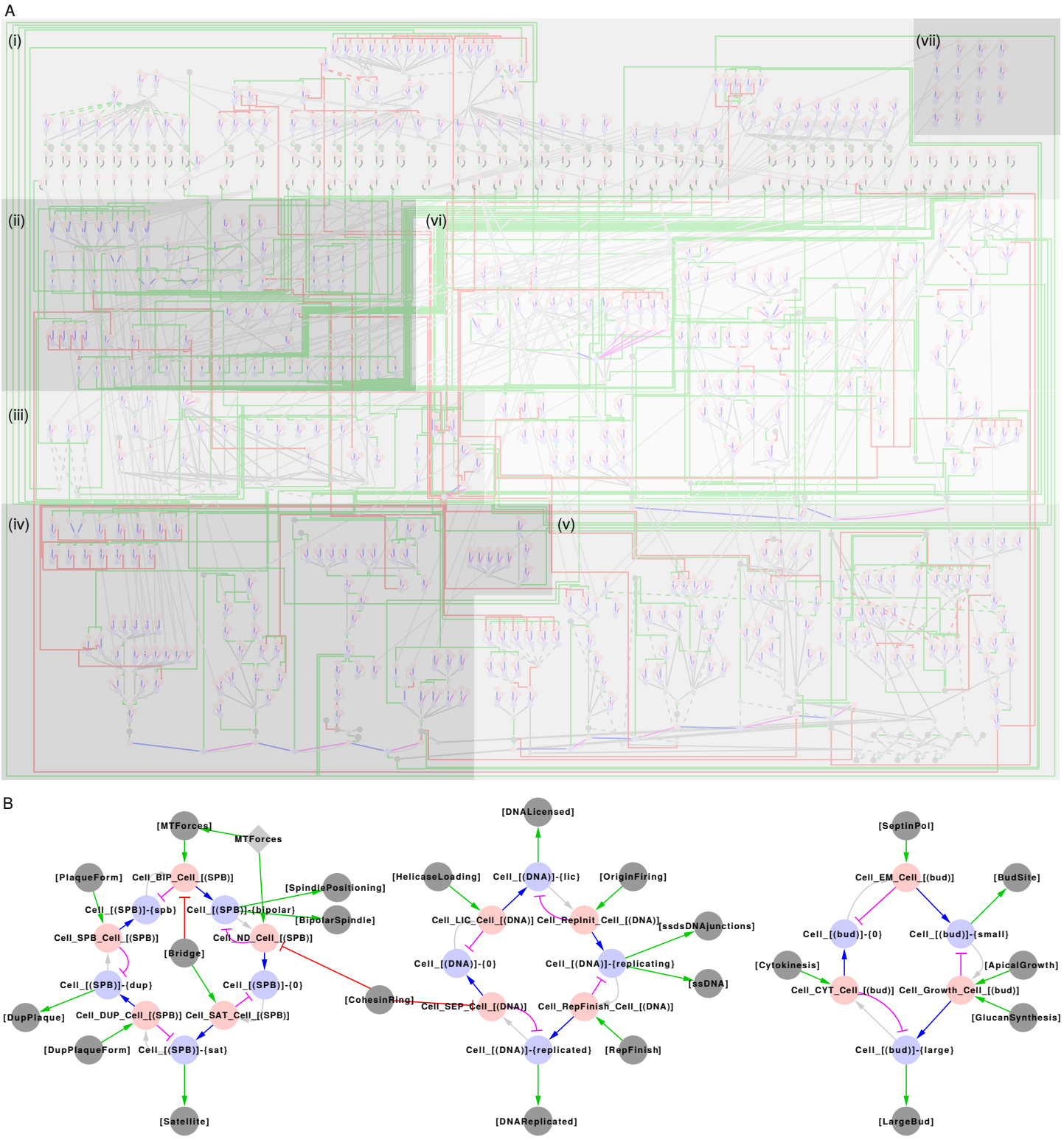
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G2/M
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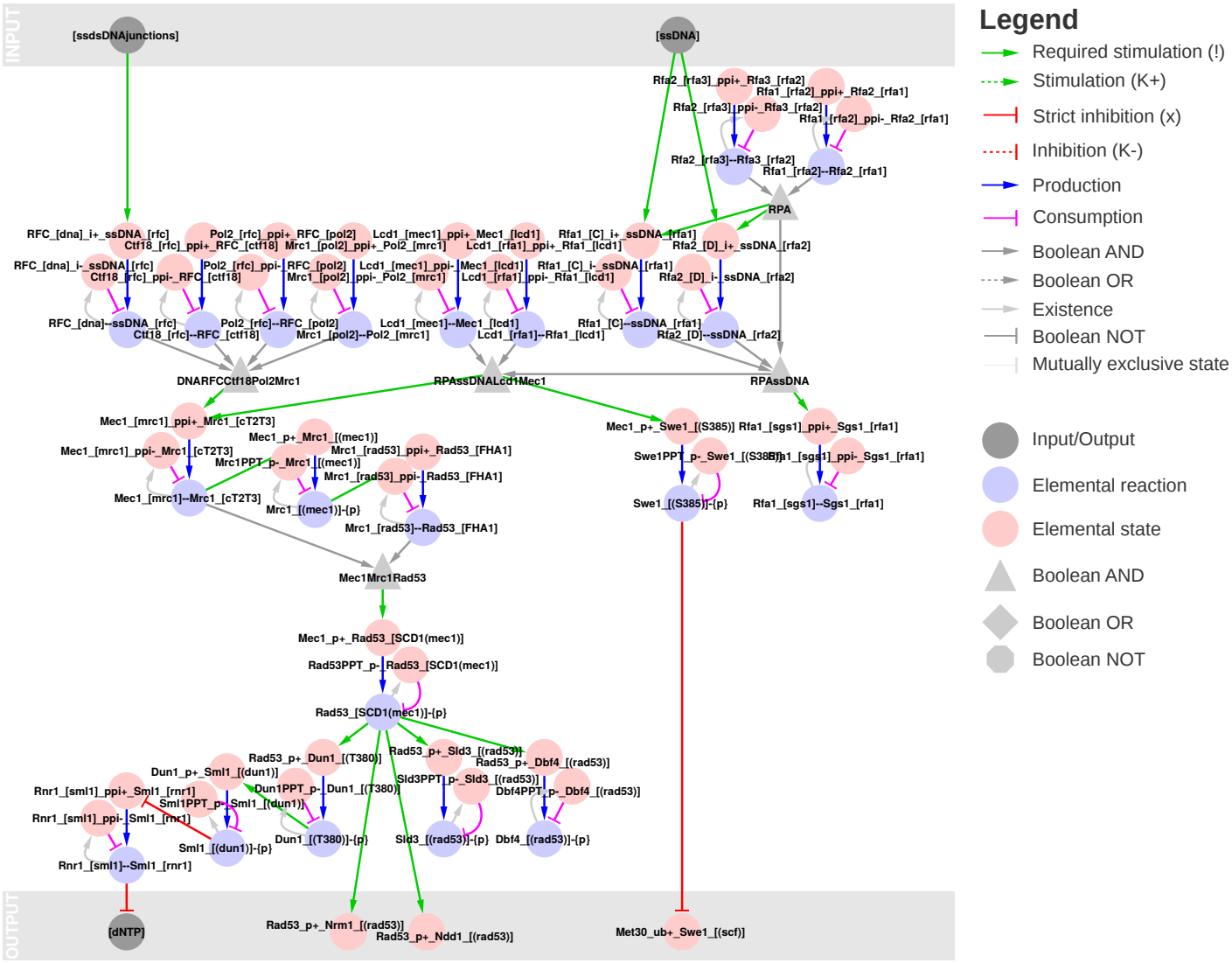
M/G1
Spindle
Spindle alignment

Figure 1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Inhibition (K-)
Production
Consumption
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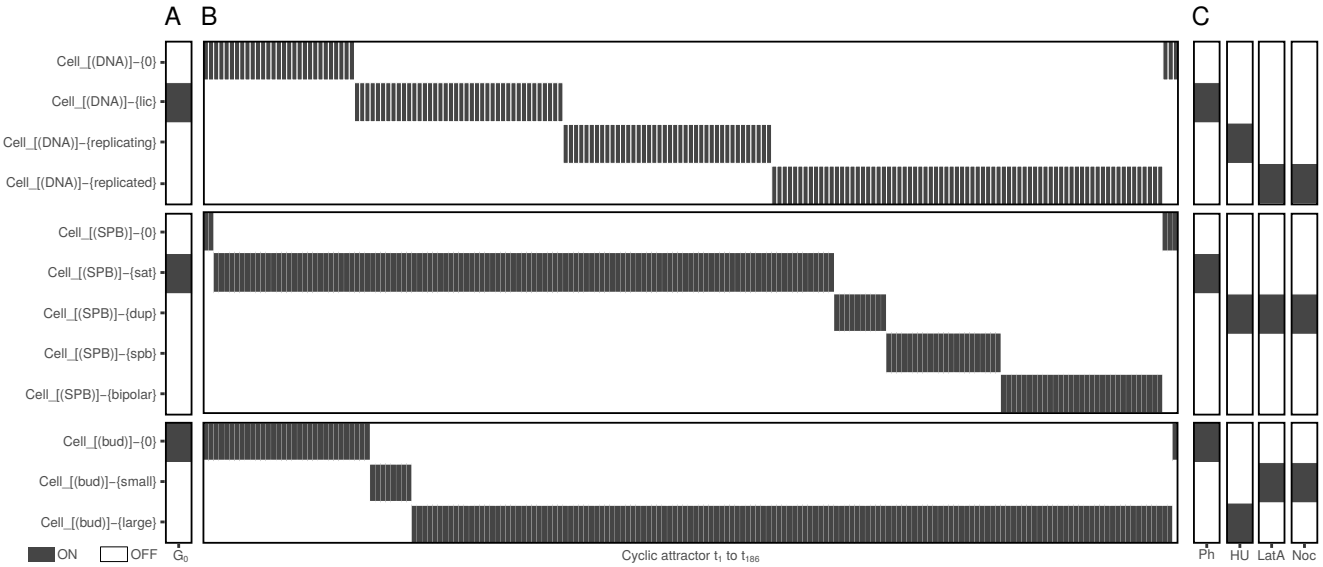
Existence
Boolean NOT
Mutually exclusive state
Input/Output
Elemental reaction
Elemental state
Boolean AND
Boolean OR
Boolean NOT

Figure 2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OFF
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Ph
HU
LatA
Noc

Figure 3

A

	Lethal	Viable
Cyclic	0	19
Point	43	23

B

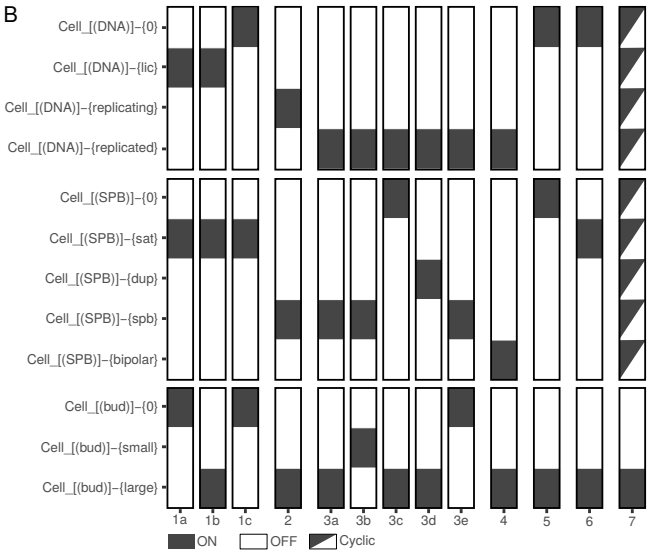

Figure 4

TABLE 1

Observation	Solution	Reference
MBF target genes constitutively expressed	Swi6 phosphorylation strict requirement for MBF activity	Palumbo et al. (2016)
Fkh2, Ndd1, Mcm1 target genes constitutively expressed	Fkh2 phosphorylation strict requirement for Fkh2 activity	Pic-Taylor et al. (2004)
Clb2 and Clb5 not degraded in G1	Ubiqitylation by both APC-Cdc20 and APC-Cdh1	Wäsch and Cross (2002), Lu et al. (2014)
Pcl9 degradation not regulated	Regulated degradation equivalent to Pcl1,2 degradation	Hernandez-Ortega et al. (2013)
Pho85-Pcl9 phosphorylation of Whi5 dominates Cdc28-mediated Whi5 phosphorylation	Pho85-Pcl9 phosphorylation positive influence on Whi5 dissociation	*Hypothesis: Alternatively, Pcl9 expression could be nutrient dependent as for Cln3*
Sic1 deactivation premature	Phosphorylation restricted to Cdc28-Cln1,2	*Hypothesis: No Cdc28-Cln3 phosphosylation of Sic1*
Bud growth module regulation insufficient	Symmetry breaking included as output/input when criteria for polarisation are fulfilled	*Placeholder for quantitative phenomenon. Cannot be described mechanstically with Boolean logic*
Stable complexes in SPB cycle disappeared	[Daughter SPB] present with duplicated and/or separated SPBs	*Technical correction*
Periodic Rad53 transcription and constant degradation lead to Rad53 periodic depletion	Constant Rad53 expression: MBF regulation as positive influence instead of strict requirement	*Hypothesis: Basal Rad53 expression constitutive to account for constant role in DNA damage response*
Discrepancy between Boolean and physiological time	Introduction of delays on transcriptional reactions	*Time scale separation. Solution to technical issue with Boolean networks.*
Dbf2 release premature	Correct spindle positioning blocks phosphorylation of Kin4 by Elm1	Consistent with SPOC activation of phosphorylation; Kurtulmus et al. 2010
No SPB duplication due to premature Spc42 disappearance. MBF transcription very brief.	Introduction of delays on replication	*Time scale separation. Solution to technical issue with Boolean networks.*
SPB positioning premature	Kar9 phosphorylation by Cdc28-Clb1,2,5,6	*Hypothesis*
No (morphological) cell division cycle due to premature activation of APC (due to Mad2 depeletion).	Degradation of Cdc20 block when bound to Mad2	*Hypothesis*
No (morphological) cell division cycle: Cytokinesis doesn't trigger as symmetry breaking and Chs2 release never overlap	Acm1 phosphorylation completely blocks (x) instead of decreases (k-) Acm1 degradation	*Hypothesis*

TABLE 2

Mutant	Arrest point	DNA	Nuclei	Comment	Synthetic Lethality
cln3	lic, sat, 0	1	1	G1-arrest	*cln3bck2*
cln3mbp1	lic, sat, 0	1	1	G1-arrest	*cln3bck2*
cln3swi6	lic, sat, 0	1	1	G1-arrest	*cln3bck2*
cln3swi4	lic, sat, 0	1	1	G1-arrest	*cln3bck2*
swi4	lic, sat, large	1	1	G1-arrest	
swi6	lic, sat, large	1	1	G1-arrest	
swi64A	lic, sat, large	1	1	G1-arrest	
mbp1	lic, sat, large	1	1	G1-arrest	
clb5	lic, sat, large	1	1	G1-arrest	*clb3clb4clb5*
cdc20pds1clb5	lic, sat, large	1	1	G1-arrest	*clb3clb4clb5*
cdc20pds1clb5cdh1SIC1	lic, sat, large	1	1	G1-arrest	*clb3clb4clb5*
spc1102ACDK	lic, sat, large	1	1	G1-arrest	
Sld2A	lic, sat, large	1	1	G1-arrest	
Sld3T600A	lic, sat, large	1	1	G1-arrest	
Sld3S622A	lic, sat, large	1	1	G1-arrest	
Sic17A	lic, sat, large	1	1	G1-arrest	
cdh1	0, sat, 0	1	1	G1-arrest	
cln1cln2cdh1	0, sat, 0	1	1	G1-arrest	
spc1104A	rep'd, dup, large	1	1	G2/M-arrest	
pds1	0, 0, large	2	2	T-arrest	
net1cdc15cdh1	0, sat, large	2	2	T-arrest	
cdc15net1	?, ?, large	*	*	Multinucleate: DNA replication and ND, no cell division	
tem1net1	?, ?, large	*	*	Multinucleate: DNA replication and ND, no cell division	

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Table S3

Mutant	Prediction	Arrest point (Fig 4B)	DNA	Nuclei
bub2	viable	N/A		
cdc28Y19F	viable	N/A		
CDC5	viable	N/A		
cdc55	viable	N/A		
clb1	viable	N/A		
clb2	viable	N/A		
clb6	viable	N/A		
cln1	viable	N/A		
cln1cln2	viable	N/A		
cln2	viable	N/A		
cln3bck2whi5	viable	N/A		
cln3whi5	viable	N/A		
lte1	viable	N/A		
spc1102AMps1	viable	N/A		
ssa1	viable	N/A		
whi5	viable	N/A		
whi512A	viable	N/A		
whi512Acln3	viable	N/A		
ydj1	viable	N/A		
cdc28	inviable	1a	1	1
cdc28T169A	inviable	1a	1	1
cln1cln2cln3	inviable	1a	1	1
cln1cln2cln3cdh1	inviable	1a	1	1
cln1cln2cln3sic1	inviable	1a	1	1
cln1cln2cln3whi5	inviable	1a	1	1
cln3	inviable	1a	1	1
cln3bck2	inviable	1a	1	1
cln3bck2sic1	inviable	1a	1	1
cln3mbp1	inviable	1a	1	1
cln3swi4	inviable	1a	1	1
cln3swi6	inviable	1a	1	1
whi54Aswi6S4A	inviable	1a	1	1
bck2swi6sic1	inviable	1b	1	1
cdc20pds1cdh1SIC1	inviable	1b	1	1
cdc20pds1clb5	inviable	1b	1	1
cdc20pds1clb5cdh1	inviable	1b	1	1
cdc20pds1clb5cdh1SIC1	inviable	1b	1	1
cdc34	inviable	1b	1	1
cdc4	inviable	1b	1	1
cdc53	inviable	1b	1	1
cdc7	inviable	1b	1	1
clb5	inviable	1b	1	1
clb5clb6	inviable	1b	1	1
cln1cln2swi4	inviable	1b	1	1
mbp1	inviable	1b	1	1
Sic17A	inviable	1b	1	1
Sld2A	inviable	1b	1	1
Sld32A	inviable	1b	1	1
Sld3S622A	inviable	1b	1	1
Sld3T600A	inviable	1b	1	1
spc1102ACDK	inviable	1b	1	1
swi4	inviable	1b	1	1
swi4mbp1	inviable	1b	1	1
swi4mbp1BCK2	inviable	1b	1	1
swi4swi6	inviable	1b	1	1
swi4swi6sic1	inviable	1b	1	1
swi6	inviable	1b	1	1
swi64A	inviable	1b	1	1
whi512Aswi6S4A	inviable	1b	1	1
cdh1	inviable	1c	1	1
cln1cln2cdh1	inviable	1c	1	1
clb1clb2	inviable	3a	1	1
NOC_CDC5	inviable	3a	1	1
HU_CDC5	inviable	3a	1	1
cdc31	inviable	3c		
spc1104A	inviable	3d	1	1
cdc10	inviable	3e	1	1
cdc11	inviable	3e	1	1
cdc12	inviable	3e	1	1
cdc24	inviable	3e	1	1
cdc3	inviable	3e	1	1
cdc42	inviable	3e	1	1
cdc14	inviable	4	1	1
cdc14SIC1	inviable	4	1	1
cdc20	inviable	4	1	1
cdc5	inviable	4	1	1
esp1	inviable	4		
cdc20pds1	inviable	5	1	1
pds1	inviable	5	2	2
cdc6	inviable	6	1	1
net1cdc15cdh1	inviable	6	2	2
cdc15	inviable	7	*	*
cdc15net1	inviable	7	*	*
tem1	inviable	7	*	*
tem1net1	inviable	7	*	*