Unifying the mechanism of mitotic exit control in a spatio-temporal logical model

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1 Abstract

The transition from mitosis into the first gap phase of the cell cycle in budding yeast is controlled by the Mitotic Exit Network (MEN). The network interprets spatio-temporal cues about the progression of mitosis and ensures that release of Cdc14 phosphatase occurs only after completion of key mitotic events. The MEN has been studied intensively however a unified understanding of how localization and protein activity function together as a system is lacking. In this paper we present a compartmental, logical model of the MEN that is capable of representing spatial aspects of regulation in parallel to control of enzymatic activity. Through optimization of the model, we reveal insights into role of Cdc5 in Cdc15 localization and the importance of Lte1 regulation in control of Bfa1. We show that our model is capable of correctly predicting the phenotype of ~ 80% of mutants we tested, including mutants representing mislocalizing proteins. We use a continuous time implementation of the model to demonstrate the role of Cdc14 Early Anaphase Release (FEAR) to ensure robust timing of anaphase and verify our findings in living cells. We show that our model can represent measured cell-cell variation in Spindle Position Checkpoint (SPoC) mutants. Finally, we use the model to predict the impact of forced localization of MEN proteins and validate these predictions experimentally. This model represents a unified view of the mechanism of mitotic exit control.
2 Introduction

The ordering of mitotic events is tightly controlled in eukaryotes in order to ensure accurate chromosome segregation and prevent aneuploidy, a hallmark of cancer (Santaguida and Amon [2015]). The MEN is a signalling network in *S. cerevisiae* that interprets spatial and temporal signals in late mitosis, ensuring mitotic exit and cytokinesis occur only after proper segregation of the genetic material (Reviewed in Rock and Amon [2009], Weiss [2012], Hotz and Barral [2014], Ibrahim [2015], Scarfone and Piatti [2015]). Since the network was first described (Jaspersen et al. [1998]), over 100 papers have been published on the topic. This volume of research has driven the MEN to become one of the best understood signalling pathways however it also poses a challenge to synthesise this knowledge. In this article we propose a compartmental, logical model of the MEN that aims to represent a unified view of the network and make predictions about its behaviour.

Progression of the cell cycle in eukaryotes is controlled by the activity of Cyclin-Dependent Kinase (CDK). CDK activity begins low in G1 phase before increasing as cells enter S-phase and reaches its peak in mitosis (Weiss [2012]). In order for the cell to complete the cell cycle and return to its G1 state, it must reduce CDK activity and reverse phosphorylation of its substrates. In yeast, unlike other model eukaryotes, this occurs in a two-step process (Ibrahim [2015]). Firstly, some cyclins, including Clb5 and to a lesser extent Clb2 (Wäsch and Cross [2002]), are destroyed at the metaphase-anaphase transition through the activity of the Anaphase-Promoting Complex (APC) in its Cdc20-coupled isoform. Then at exit from mitosis, the phosphatase Cdc14 is released from the nucleolus and de-phosphorylates multiple CDK phosphosites around the cell, in particular Cdh1, an alternative APC subunit capable of targeting mitotic cyclins for destruction. This leads to complete reversal of mitotic CDK phosphorylation, permitting division of mother and daughter cells, and returns the cell to a G1 state.

This two-step process of CDK destruction in mitosis is required in budding yeast as its morphology necessitates two checkpoints rather than the one normally found in eukaryotic cells. The Spindle Assembly Checkpoint (SAC) is found in most eukaryotes and ensures the kinetochores are correctly attached to chromosomes before spindle elongation occurs and acts through control of the APC subunit Cdc20. In contrast, the Spindle Position Checkpoint (SPoC) is not present in most eukaryotes. The SPoC acts to ensure both mother and daughter cells receive the full complement of genetic material after cytokinesis, by delaying mitotic exit until alignment of the spindle with the mother-bud axis.

The two-step process of CDK inactivation is mirrored by the regulation of Cdc14 localization. Throughout the majority of the cell cycle, Cdc14 remains tightly sequestered in the nucleolus, through its interaction with Net1. In early anaphase, Cdc14 is released into the nucleus by a group of proteins known as the FEAR network (Figure 1). This release is not sufficient to initiate exit from mitosis, and in fact is transitory as Cdc14 will return to the nucleolus if the SPoC remains in place (Stegmeier et al. [2002]). MEN activity, unlike the FEAR network, leads to sustained release of Cdc14, which in turns
activates the alternative APC subunit, Cdh1, and the CDK inhibitor, Sic1, leading to cytokinesis and the entry of the cell into a new reproductive cycle (Weiss [2012]). The MEN is controlled by both the SPoC and indirectly by the SAC, as the high level of CDK activity in metaphase prevents MEN activation.

Control of localization is key to the regulation of the MEN. MEN-activating factors, such as Lte1, reside in the bud, while MEN-inhibiting proteins like Kin4 are restricted to the mother compartment (Bardin et al. [2000], Pereira and Schiebel [2005]). Alignment of the spindle with the mother-bud axis is signalled by the entry of a Spindle Pole Body (SPB) into the bud (Scarfone and Piatti [2016, 2015]). MEN proteins localize to the SPB and so sense the change from the mother to the bud compartment, leading to MEN activation. The MEN pathway culminates in activation of the Mob1-Dbf2 kinase complex (Figure 1). The exact mechanism by which Mob1-Dbf2 promotes mitotic exit is not yet fully understood, however it is known that the complex enters the nucleus (Stoepel et al. [2005]), phosphorylates Net1 (Ptacek et al. [2005]) and phosphorylates Cdc14 near to its NLS, allowing Cdc14 to leave the nucleus (Mohl et al. [2009]).

The volume of research into mitotic exit makes it difficult to provide a unifying view of the process using only informal models, and so we turn to formal mathematical models which can account for this complexity. There are a number of published mathematical models of mitotic exit in budding yeast however none represent the full extended network with spatial detail. Some of these are comprehensive Ordinary Differential Equation (ODE) models of the cell cycle, which include some details of the MEN, for example Chen et al. [2004] and Kraikivski et al. [2015]. While these models represent some aspects of mitotic exit control, their broad scope means they lack detail. The Queralt model (Queralt et al. [2006], Tóth et al. [2007]) was the first mathematical model built to focus on the regulation of Cdc14. This work was used as a basis for further modelling, with Vinod et al. [2011] and Hancioglu and Tyson [2012] expanding the Queralt model to examine the interplay between the MEN and the FEAR network. These ODE models were important to develop the biological understanding of the MEN however they are unable to represent spatial regulation. A compartmental ODE model of the SPoC was used by Caydasi et al. [2012] to explore the spatial aspects of Tem1 and Bub2-Bfa1 regulation, however its scope was limited to a small number of proteins. We aim to build on these models to create a model of the extended network in spatial detail.

A major limitation of ODE models is their scalability; a large ODE model requires many parameters which must be experimentally measured or inferred, which is virtually impossible for larger networks. Logical models are an alternative modelling formalism which attempts to avoid the issue of parameterization by focusing on network structure, producing qualitative, binary predictions about activity of proteins (Reviewed in Bornholdt [2008], Abou-Jaoudé et al. [2016]). Generalized logical models are an extension of the Boolean formalism; the nodes of a logical model may take any number of discrete states, instead of the two (0 or 1) permitted in Boolean models. Many tools for the construction and simulation
of logical models have been developed, with an emphasis on interoperability (CoLoMoTo.org). Logical models have been used widely to explore cell cycle regulation in yeast, starting with Li et al. [2004], and some large scale models have included coarse-grained representations of exit from mitosis (Irons [2009], Fauré et al. [2009]). A logical model was used to explore regulation of the Septation Initiation Network (SIN) (Chasapi et al. [2015]), the MEN’s homologous pathway in S. pombe (Bardin and Amon [2001]). Münzner et al. [2019] recently published the most comprehensive logical yeast cell cycle model yet. However, this model does not explicitly represent spatial effects in the network, and 3 out of 12 genetic phenotypes relating to core MEN proteins that the authors tested were found not to match the experimentally determined phenotypes. Some variations of the logical modelling formalism have included spatial effects, for example the EpiLog tool (Varela et al. [2018]), which models cell-cell signalling. However, there is currently no logical formalism capable of representing intracellular spatial regulation. To overcome these limitations we have created a novel compartmental logical modelling framework that can represent spatial effects.

We used this compartmental logical modelling framework to build a model of mitotic exit control. Our proposed model aims to represent the above aspects of regulation of Cdc14 localization from metaphase to telophase. In recent years, further roles for the MEN have been proposed in the control of spindle alignment (Hotz et al. [2012]) and in cytokinesis (Tamborrini et al. [2018]), however we consider these processes to be beyond the scope of the model. Furthermore, we model only the process of initiation of mitotic exit and not its execution, so phenomena such as Cdc14 endocycles that rely on degradation of Cdc5 during mitotic exit (Lu and Cross [2010], Manzoni et al. [2010]) are outside the scope of the model.

3 Methods

3.1 Logical Modelling

In the logical modelling formalism, proteins are nodes, with edges between nodes representing their regulation (activation or inhibition) (Abou-Jaoude et al. [2016]). Therefore, a logical model is a directed, signed network along with a set of logical (ANDs and ORs) rules describing how the different kinds of regulation interact with each other (Figure 2B). Any logical rule may be written in disjunctive normal form, a single OR over multiple ANDs. This simplifies interpretation of the rule and so as far as possible we have used this representation. The final ingredient required for a logical model is an update scheme, describing when nodes have their state updated. The simplest update scheme is a synchronous scheme where all nodes are updated at the same time. Attractors are sets of network states which, once entered, cannot be left under the synchronous update scheme. The simplest attractors are steady states, which are states that once entered, the model will never leave. There are also higher order attractors containing multiple states; a model in such a state will cycle through multiple intermediate states in a given order.
It can be shown that all logical networks have at least one attractor. While the synchronous update scheme is simple, it is unrealistic, as in real biochemical systems, some reaction will always occur first (Abou-Jaoudé et al. [2016]). Therefore, asynchronous update schemes, in which nodes are updated independently in a predefined or random order, give more realistic dynamics. Any steady state under the synchronous update scheme will remain steady under an asynchronous update scheme although this is not always true of higher order attractors.

3.2 Compartmental logical modelling

In a compartmental ODE model, the concentration of each protein in each compartment is described by separate variables. Similarly in a compartmental logical model, a node exists for each protein in each compartment it is permitted to localize to. The state of this node corresponds to whether the protein is present in this compartment (1) or not (0). In addition to these localization nodes, an activity node for each protein and each compartment exists to track whether the protein is active in this compartment (1) or not (0). Activation of the localization node is a pre-requisite for activation of the activity node. The rules of the network are then built from an activity network - describing how proteins control each others enzymatic activity through post-translational modifications - and a localization network - describing how proteins control each others localization to different compartments. The resulting network can be expressed as a logical network, albeit one where each protein appears multiple times for each compartment. This means that compartmental logical networks are larger than the underlying networks. If there are $n$ proteins represented in the model and $C$ compartments, the resulting compartmental logical network has $2 \times n \times C$ nodes.

3.3 Model construction

The FEAR network was trained using the CellNOptR package (Terfve et al. [2012]), run 100 times in parallel, with max inputs per gate set to 4. Activity and localization networks as well as a set of location specific rules were created in BoolNet format and custom R scripts were used to generate the compartmental logical model. Briefly, the relevant nodes were created for each of the permitted locations and then the rules were read in and distributed across the nodes by creating an edge list. This edge list included modifications, such as requiring localization to be on for the activity node to be switched on. Generally, protein activity can only be regulated by proteins in the same compartment. The exception is the SPB, where proteins that can localize to the SPB can be regulated by these proteins or, if their regulator cannot localize to the SPB (for example Lte1), by proteins in the surrounding cytoplasm. The “Spindle alignment” node controls whether proteins at the dSPB can be regulated or exchange with the cytoplasm (“Spindle alignment” = 0) or with the bud compartment (“Spindle alignment” = 1). All R scripts may be accessed at https://github.com/RowanHowell/CLM-R.
3.4 Simulation of mutants

To analyse the phenotypes of mutant strains, we placed all mutations into one of the following categories:

1. **Hyperactive**, an allele which is resistant to inhibition and will be active wherever it is localized.

2. **OE**, overexpression either by the *GAL1* promoter or provision of the gene on a multicopy plasmid, the protein is present and active everywhere. This high level of expression a protein can break the usual rules of protein regulation (see below).

3. **KD**, (knock-down), a functionally inactive allele, often temperature or analogue sensitive, which is inactive wherever it is localized.

4. **Delete** or **Deplete**, either deletion of the protein or depletion via a conditional promoter, localization prevented everywhere.

5. **Location** or **!Location**, the forced localization of the protein in one compartment or (!) the prevention of that localization.

6. **Phosphomutants**, phosphomutants represent a re-wiring of the network and therefore were considered on a case-by-case basis.

Of the above, most are straightforward, however overexpression can have many effects on protein function, and in some cases the sheer quantity of the overexpressed protein can alter the wiring of the network, for example activating a downstream component despite the presence of an inhibitor (Moriya [2015]). To account for these effects we introduced additional overexpression nodes for each protein. In the case where a protein activates its downstream components in a way that could be blocked by an inhibitor, the overexpression node circumvents this inhibition (Figure S1B). However, note that localization of a protein in a compartment is always a necessary requirement for that protein’s activity in that compartment.

Similarly overexpression of an inhibitor may block the activation of a protein even in the presence of an activator. The only exceptions to this are the cases of non-physiological conditions of high Tem1 activity and lack of low level of Bub2 and Bfa1 activity in models 3 and higher. These modifications were applied to the edge list described above, and were used to produce a variant of the model, usually suffixed “OE”.

Similarly, we treated forced localization at the SPB as equivalent to a local overexpression, so a model variant suffixed “SPB” included the forced localization nodes needed to model this perturbation.

3.5 Discrete time dynamics and steady states

We represented the logical model as a Boolean network, in which each level of activity is represented as an individual node, in order to make use of computational tools designed for Boolean networks. As synchronous steady states are necessarily steady states in an asynchronous setting, all steady states were
identified on the synchronous model. This was performed by solving the satisfiability problem using the BoolNet package for R (Müssel et al. [2010]), which employs the PicoSAT solver (Biere [2008]), based on the algorithm of Dubrova and Telsenko (Dubrova and Teslenko [2011]). In some cases multiple steady states exist for the same cell cycle stage, in order to determine the phenotype in such situations we used Monte Carlo simulations of the asynchronous model with nodes chosen uniformly at random, again using BoolNet functions. Unless otherwise stated all simulations were performed from the same, physiological initial conditions and were ran until either steady state was reached, the “Mitotic Exit” node was activated or the number of timesteps reached 10,000. All R scripts may be accessed at https://github.com/RowanHowell/CLM-R.

3.6 Continuous time dynamics

We used the MaBoSS package (Stoll et al. [2012]), using scripts developed for the MaBoSS python package (Stoll et al. [2017]), to perform continuous time simulations of the logical model. The BoolNet model was converted to MaBoSS format using the GINsim tool (Naldi et al. [2009]). Rate parameters were fit to match experimentally determined length of mitosis in bub2Δ and kin4Δ cells (Figure S4, Falk et al. [2016a], Table S1). Spindle alignment times were simulated as a Brownian motion, using a custom Python script. All Python scripts may be accessed at https://github.com/RowanHowell/CLM-Python.

3.7 Yeast Strains & Methods

Yeast was cultured in standard growth media with 2% (w/v) glucose at 30°C, unless otherwise stated. All yeast strains are derivatives of BY4741, unless otherwise specified. Plasmids were constructed by gap repair either through in vivo recombination or the NEBuilder plasmid assembly tool (New England Biolabs, USA). Linear products were created by PCR with primers from Sigma Life Science and Q5 Polymerase (New England Biolabs, USA). The strains and plasmids used in Figure 5D were a gift from Gislene Pereira (Caydasi et al. [2017]), with the exception of the empty plasmid, which was pWJ1468. mRuby2-TUB1 strains were constructed using a linearized plasmid, “pHIS3p:mRuby2-Tub1+3’UTR::URA3”, which was a gift from Wei-Lih Lee (Addgene plasmid # 50639; http://n2t.net/addgene:50639; RRID:Addgene_50639) (Markus et al. [2015]). NUD1-GFP strains are derived from a library derived from BY4741 (his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) (Huh et al. [2003]; Tkach et al. [2012]). TEM1-YFP and CDC15-YFP strains were constructed by homologous recombination from E438. GBP and CLB2-CDC28-GBP plasmids express their product from the the MET3 promoter and were derived from pWJ1512 (Reid et al. [2011]). All spot assays show 10 fold serial dilutions, with cultures of the same optical density. Counter-selection of plasmids with uracil selection was achieved by addition of 750µg/ml 5FOA (FluoroChem Ltd). Plasmids used in this study are listed in Table S2, strains are listed in Table S3.
3.8 Fluorescence microscopy

In the anaphase length assay, cells were grown, shaking, overnight in synthetic complete (SC) media at 30°C, then diluted 1 in 10 in fresh SC media and left to grow for 3 hours. Cells were then transferred to a -uracil agarose cube and placed into a sealed chamber. *SPO12* and *spo12*∆ cells were placed in side-by-side chambers. The cells were pre-incubated at 30°C for an hour before imaging. Cells were imaged using a DeltaVision®Elite (GE Healthcare), with a 60x 1.42NA Oil Plan APO and an InsightSSI 7 Colour Combined Unit illumination system (CFP = 438nm, mRuby2 = 575nm). Images were captured with a front illuminated sCMOS camera, 2560 x 2160 pixels, 6.5µm pixels, binned 2x2. Time lapse videos were captured over 2 hours, with images captured at 2 minute intervals. Images were analysed using FIJI (Schindelin et al. [2012]), with the Bio-formats plugin (Linkert et al. [2010]). For the forced CDK localization experiments, cells were grown shaking overnight in -leucine media supplemented with additional methionine at 23°C. They were then transferred to -leucine -methionine media and grown shaking for 4 hours at 23°C and then imaged. For the *NUD1-GBP* forced localization experiments, functionality of the SAC was assessed as described in Fraschini [2016]. Cells were grown shaking overnight in 2% raffinose -leucine media. They were then transferred to 2% raffinose -leucine media for 2 hours before being spun down and washed in water. They were resuspended in 2% galactose YP media containing 3µg/ml alpha factor. They were transferred to incubate shaking for 2 hours before the alpha factor (The Francis Crick Institute Peptide Chemistry STP) was washed out and the cells were washed and resuspended in 2% galactose YP media containing 15µg/ml nocodazole (Sigma-Aldrich) and incubated shaking for 3 hours. In both assays cell were imaged with a Zeiss Axioimager Z2 microscope (Carl Zeiss AG, Germany), with a 63x 1.4NA oil immersion lens and using a Zeiss Colibri LED illumination system (RFP = 590 nm, GFP = 470 nm). Bright field images were obtained and visualized using differential interference contrast (DIC) prisms. Images were captured using a Hamamatsu Flash 4 Lte. CMOS camera containing a FL-400 sensor with 6.5 µm pixels, binned 2x2. Images were analysed with ICY (de Chaumont et al. [2012]).

3.9 ODE simulations

Simulations of the ODE model of Caydasi et al. [2012] (BioModels database ID: BIOMD0000000702) were performed with Copasi (Hoops et al. [2006]), using the CoRC package. Parameters were unchanged from the original model, except initial conditions which were chosen to match the steady states of the pre-alignment model. Forced localization of Bfa1 at the SPB was modelled by decreasing the off-rate of Bfa1 species by a factor of 1,000.
4 Results

4.1 Model construction

Due to the complexities of the spatial aspects of the model we combined an expertise-based approach with a model-fitting approach to construct the model. The FEAR network, which acts only in the nucleus was trained against a dataset of 50 mutant phenotypes using the CellNOptR tool (Terfve et al. [2012]). The rest of the model was built from the literature, and the trained FEAR network was integrated into it.

CellNOptR uses a genetic algorithm to train a Boolean model against known phenotypes (Saiz-Rodriguez et al. [2009], Terfve et al. [2012]). The genetic algorithm takes a Prior Knowledge Network (PKN) and evolves this network, using its fit to data as a measure of fitness, to optimise the model with respect to the dataset. We built a PKN comprising of 22 edges (Supplementary File 1) and trained it against a dataset of 52 mutants from 11 publications (Supplementary File 2), using the early anaphase release of Cdc14 from the nucleolus as the output. A detailed, referenced description of the FEAR network can be found in the Supplementary Information. We found that, due to the stochasticity of the genetic algorithm, there was significant variation between the fit achieved by independent runs of the algorithm. For this reason, we ran the algorithm 100 times, distributed in parallel and considered the optimal fits achieved. We found that several phenotypes, in particular those relating to Cdc5 overexpression, were difficult for the algorithm to fit. Cdc5 when expressed at a high level is clearly capable of releasing Cdc14 (see for example Visintin et al. [2003]), however the activity of Cdc5 is thought to be stable throughout late mitosis, suggesting it is not part of the temporal signal initiating FEAR release. Therefore, we reasoned that overexpression is likely to break the normal logic of Net1 inactivation. For this reason we allowed overexpression of Cdc5 to “feed-forward” and regulate Net1 according to different rules than those for physiological levels of Cdc5. Practically, this meant we introduced a node called “Cdc5OE” which had the same outputs as Cdc5 in the PKN, this node was then treated as all the others in the training process. We found this allowed for the identification of models which could fit 88% of the training dataset. The “Cdc5OE” node was removed during integration with the MEN model but its effect was recovered by the addition of overexpression nodes for each protein in the model.

A compartmental model is the product of an activity and localization network superimposed (Figure 2C). We could not enforce the necessary conditions to use CellNOptR to train this kind of model, although in principle an evolutionary algorithm could be used for this purpose. Instead we decided to build the model by hand; a list of update rules for each node in the final model (Model 5) with details of the evidence for each can be found in Supplementary File 3 and a graphical representation is given in Figure S1A. A detailed, referenced description of the MEN can be found in the Supplementary Information. The model has five compartments: the nucleus, cytoplasm (mother compartment), bud,
mSPB and dSPB (Figure 2A). In budding yeast, the old and new SPBs have some minor physiological differences and it is the old SPB that enters the bud (Pereira et al. [2001]). However, it has been shown that reversing this pattern has no significant effect on MEN signalling (Manzano-López et al. [2019]) and therefore we consider the dSPB and mSPB to refer only to the destination of the SPB and not to their age-related identity.

A key decision was how to model the activities of CDK and Cdc14. CDK activity depends on the concentration of cyclins in the cell, early mitotic and S-phase cyclins such as Clb5 are largely degraded by the Anaphase-Promoting Complex (APC) in its Cdc20 isoform at the metaphase-anaphase transition. Consequently, the level of CDK activity in the cell decreases, however late mitotic cyclins, such as Clb2, remain present until activation of the alternative APC subunit, Cdh1, at mitotic exit. In the interests of simplicity we do not distinguish specific cyclin contributions, instead the model has a high and low level of activity for CDK, representing the metaphase and anaphase levels of CDK respectively. Similarly, the Cdc14 nodes are two-levelled, as although FEAR release of Cdc14 is largely limited to the nucleus, its impact on MEN proteins means that a low level of Cdc14 must reach the cytoplasm in early anaphase. We also used two levels for Cdc15 nodes. There is limited evidence that the activity of Cdc15 changes throughout mitosis, instead Cdc15 is thought to be controlled by its localization, with geometric constraints and enzymatic funnelling allowing Cdc15 to phosphorylate Dbf2 only at the SPB. However, Cdc15 and CDK are thought to engage in a negative feedback loop, where Cdc15 phosphorylation of Nud1 allows CDK localization at the SPB, which itself prevents Cdc15 localization, closing the loop (König et al. [2010]). The localization of active Tem1 at the SPB, then allows a high level of Cdc15 to localize to the SPB, leading to recruitment of Mob1 and activation of Dbf2. For reasons not yet understood, CDK is excluded from the SPB in the presence of active MEN components. We represented this effect by inclusion of a rule preventing CDK localization at the SPB in the presence of active Mob1 and Dbf2. Therefore, the model includes a low level of Cdc15 capable of engaging in the Cdc15-Nud1-CDK feedback loop, and a high level that is recruited by Tem1 and can activate Mob1-Dbf2. We modelled the activity of Kin4 as preventing Bfa1 localization at the SPB, this is a simplification of the real mechanism in which Kin4 phosphorylation increases the turnover of Bfa1 at the SPB (Caydasi and Pereira [2009]). Both of these mechanisms have the same effect of keeping Bfa1 away from its inhibitor Cdc5 at the SPB.

We combined the FEAR and MEN models into a single compartmental logical model, treating the FEAR network as an activity network, with the exception of PP2A-Cdc55 (Rossio and Yoshida [2011]) and Cdc14 which are controlled through localization. The resulting network consists of 270 nodes and 523 edges (296 nodes and 748 edges in the model with overexpression).
Model | Modification
---|---
0 | Base model.
1 | As 0 and the low (anaphase) level of CDK inhibits Cdc15 loading in absence of Tem1.
2 | As 1 and the ASC inhibits Cdc15 loading in metaphase, in the absence of Tem1 and CDK.
3 | As 2 and multi-level Tem1, Bub2 and Bfa1.
3a | As 3 and identification of ASC as Cdc5.
4 | As 3 and Lte1 can inhibit Bfa1 activity in a mechanism parallel to Kin4.
4a | As 3 and Lte1 can activate Tem1 activity in a mechanism parallel to Bfa1 inhibition.
5 | As 4 and identification of ASC as Cdc5 and above.
6 | As 5 but Lte1 regulation of Bfa1 can influence speed of Tem1 activation (MaBoSS implementation).

Table 1: Description of model versions

### 4.1.1 Restriction of mitotic exit to anaphase

While developing the model, we tested it against a number of well-characterised mutants in order to refine its behaviour. In particular we found that fitting phenotypes relating to the restriction of mitotic exit to anaphase posed a challenge and necessitated further development of the model. The MEN can be thought of as a coincidence detector, waiting for both temporal and spatial signals before allowing mitotic exit to occur (Rock and Amon [2011], Caydasi et al. [2017], Campbell et al. [2019]). However, there is still contention over how exactly MEN activity is restricted to anaphase. Two classes of mutants show MEN activity prior to anaphase: firstly \( bfa1 \Delta \) or \( bub2 \Delta \) (Fraschini et al. [1999]) and secondly \( CDC15-7A \), \( MOB1-2A \) (Jaspersen and Morgan [2000], König et al. [2010], Campbell et al. [2019]). These mutants differ in that deletion of \( BUB2 \) or \( BFA1 \) renders the MEN completely insensitive to spatial or temporal signals. On the other hand, the \( CDC15-7A \) mutation results in disruption of the SPoC (Falk et al. [2016a]) and when combined with the \( MOB1-2A \) mutation will exit mitosis at any point in anaphase, or upon spindle alignment in metaphase (Campbell et al. [2019]). Initially, we tested our model against the phospho-null mutants by creating new networks in which edges joining CDK and Cdc14 to Cdc15 and Mob1 were removed.

Our original model (Model 0) could not fit the behaviour of the \( CDC15-7A \) mutation (Figure 3). This was because this model relied on localization of Tem1 at the SPB to allow full recruitment of Cdc15 there, meaning that disruption of the CDK regulation of Cdc15 would not change the spatial signalling. To address this we created a new model (Model 1), in which Cdc15 could localise to the SPB in the absence of CDK. This model could now represent the behaviour of \( CDC15-7A \) cells but not the \( CDC15-7A, MOB1-2A \) double mutant (Figure 3.). Model 1 predicts that the double mutant should exit mitosis at any point during metaphase, whereas the correct behaviour in metaphase is to wait until spindle alignment (Campbell et al. [2019]). This points to an additional level of regulation of Cdc15. We propose that Cdc15 can load onto SPBs in the absence of CDK or Tem1, in a way that is dependent on an Anaphase Specific Component (ASC). We introduced the ASC into a further model (Model 2) and
found that this model is now capable of correctly representing the behaviour of the single and double phospho-mutants (Figure 3).

Having established that the model requires both CDK inhibition of Cdc15 localization at the SPB and an additional level of regulation from an unknown ASC, we decided to test whether the model could predict the phenotype of \( bfa1^{\Delta} \). We found that Model 2 predicted that \( bfa1^{\Delta} \) cells would exit mitosis in anaphase regardless of spindle alignment, but not in metaphase (Figure 3). This is because in this model Cdc15 and Mob1 are still inhibited by CDK. In order for the disruption of the upstream MEN components like Bfa1 to affect Cdc15 and Mob1, it is necessary for this state to be transmissible. Therefore, we introduced two levels of activity for Bub2, Bfa1 and Tem1 (Model 3). In our model of a wild type cell, Bub2 and Bfa1 vary from a high level of activity, in which Tem1 is fully inhibited, to a low level, where Tem1 is sufficiently active to recruit Cdc15. When modelling \( bfa1^{\Delta} \) or \( bub2^{\Delta} \) cells, Tem1 becomes hyperactivated, allowing it to localise to the SPB independently of Bub2 and Bfa1 and recruiting Cdc15 despite the presence of high levels of CDK. This is justified by the finding that a constitutively active mutant \( TEM1^{Q79L} \) localizes symmetrically to both SPBs independently of Bfa1 (Scarfone et al. [2015]). Furthermore, in order for the hyperactivated state of Tem1 to transmit to Mob1, Model 3 allows this high level of Tem1 activity to combine with Cdc15 to recruit Mob1. Although Cdc15 can function in the absence of Tem1 (Rock and Amon [2011]), Tem1 and Cdc15 are found in complex under physiological conditions (Bardin et al. [2000], Asakawa et al. [2001]), suggesting that Tem1 may be able to play a role in recruiting Mob1. This updated model is capable of representing the behaviour of \( bfa1^{\Delta} \) and \( bub2^{\Delta} \) mutants (Figure 3).

### 4.1.2 The SPoC in the absence of Kin4

Although the \( kin4^{\Delta} \) and \( bub2^{\Delta} \) mutants both lead to loss of SPoC function, the extent of loss of function differs between the two. This can be seen both in the proportion of cells that exit mitosis with misaligned spindles and the importance of the FEAR pathway in these cells (Falk et al. [2016a]). Intriguingly, a \( kin4^{\Delta}\text{spo12}^{\Delta} \) double mutant has a fully functional SPoC (Falk et al. [2016a]), implying that spatial information is transmitted to the MEN via other mechanisms than just Kin4 activity. We tested the SPoC activity of \( kin4^{\Delta} \), \( spo12^{\Delta} \) and double mutant cells in the model and found that in Model 3 the double mutant behaved like \( kin4^{\Delta} \) (Figure 4). In Model 3, the downstream effector of the MEN-activating zone is Kin4, so in its absence there is no way for spatial information on spindle alignment to be transmitted to the MEN. A likely candidate for an additional spatial regulator of the MEN is Lte1 itself, and deletion of Lte1 in a \( kin4^{\Delta}\text{spo12}^{\Delta} \) background causes a significant delay in mitotic exit (Falk et al. [2016a]). We created a new version, Model 4, in which Lte1 could inhibit Bfa1 activity in a way that depends on the phosphorylation of Bfa1. The introduction of this additional spatial regulation of the MEN, meant that Model 4 could correctly represent both single and double \( kin4^{\Delta} \) and \( spo12^{\Delta} \)
mutants (Figure 4). It is of note that a version of the model (Model 4a) where Lte1 targets Tem1 directly could not correctly represent the phenotype of \textit{KIN4} overexpression (Figure S2).

### 4.1.3 Cdc5 is the Anaphase Specific Component (ASC)

We introduced the Anaphase Specific Component (ASC) to the model to allow for inhibition of Cdc15 localization in metaphase. One candidate for this regulation is polo kinase Cdc5, which was identified as required for localization of overexpressed Cdc15 at the SPB in the absence of Tem1 (Rock and Amon [2011]). Fluorescently-tagged Cdc5 is visible at the SPBs throughout mitosis however recent research suggests that it moves from the nuclear face of the SPB to the cytoplasmic face at the metaphase-to-anaphase transition (Botchkarev et al. [2017, 2014]). These two findings together suggest that Cdc5 could play the role of the ASC. When building Model 3 we considered Cdc5 as a candidate for the ASC (Model 3a), but found that before making the modifications in Model 4, this change prevented mitotic exit in \textit{CDC15-7A MOB1-2A} cells with aligned spindles in metaphase (Figure S2). However, the combination of identifying Cdc5 as the ASC and the regulation of Bfa1 by Lte1 from Model 4 resolves this issue (Model 5, Figure 4). Note that the identification of Cdc5 as the ASC necessitated a slight modification of the rule for Bfa1 to ensure that it can be inhibited at the SPB in metaphase. In this version of the model the essential role of Cdc5 is only in the recruitment of Cdc15 and not in Bfa1 inhibition.

### 4.2 Model validation

Having optimized the model’s behaviour against specific mutants to create Model 5, we wanted to know whether the model could accurately predict the phenotype of the many mutants described in the literature. We found 140 mutants from 37 publications, representing overexpressions, knock-downs, deletions and forced localizations of proteins present in the model. We classified these mutants depending on whether they exited mitosis in metaphase, early anaphase (before entry of an SPB into the bud) or late anaphase (after entry of an SPB into the bud). Mutants which exited mitosis in early anaphase were detected in a genetic background lacking either \textit{KAR9} or \textit{DYN1}, as these backgrounds result in a high frequency of cells with misaligned spindles. In some cases we found disagreement between papers on particular mutants, in these cases we chose a single finding to include in the dataset, prioritising experiments in the S288C/BY4741 genetic background, which were more numerous.

We simulated each of these mutants 100 times using the asynchronous update scheme and then calculated the percentage that exited mitosis, as judged by full release of Cdc14 into the cytoplasm. We simulated each mutant from realistic initial conditions (Supplementary File 4). We then determined whether the majority of simulated cells exhibited the expected behaviour for each mutant in a given condition (Supplementary File 6). We found that the model correctly predicted the phenotypes of 81% (113/140) of mutants tested (Figure 5A).
Some of the phenotypes which the model predicted incorrectly relate to the rescue of temperature sensitive alleles. For example, overexpression of Cdc5 can alleviate the temperature sensitivity of some MEN mutants, such as *tem1-3* (Jaspersen et al. [1998]), as well as cause release of Cdc14 during metaphase arrest (Hu et al. [2001]). However, while our model predicted that Cdc5 could cause unscheduled Cdc14 release, it required MEN proteins to do so and could not predict the alleviation of temperature sensitivity (Figure 5B). At an earlier point of testing, we found a similar effect with Spo12, another FEAR protein, which can rescue temperature sensitivity of MEN mutants but our model predicted otherwise. A recent study showed that although Spo12 overexpression can rescue temperature sensitive MEN mutants, it cannot rescue deletion of these proteins (Caydasi et al. [2017]). Simulation of Spo12 overexpression combined with loss-of-function MEN mutations produced results that matched the phenotype of the full deletion of these MEN proteins, rather than the temperature sensitive alleles. Altogether this suggests that Spo12 overexpression allows mitotic exit to occur at a lower, but not zero, level of MEN activity.

We suspected that a similar effect may explain the model’s inability to predict the effects of Cdc5 overexpression. To test this, we transformed a *mob1*Δ strain, kept alive by provision of a plasmid encoding MOB1, with a 2µm plasmid expressing CDC5. When the MOB1 plasmid was selected against using 5FOA, we found that the provision of CDC5 from a 2µm plasmid could not suppress the lethality of MOB1 deletion (Figure 5D), in agreement with the model’s prediction (Figure 5C). This demonstrates that any effect caused by Cdc5 overexpression must rely on activation of at least the final part of the MEN pathway. More broadly, it suggests suppression of partial or conditional lethality is a particular issue for our logical model, in which activity must be set to one of a number of discrete states.

We found that many of the phenotypes which the model predicted incorrectly relate to overexpression (19/27), especially when combined with other mutations (18/27). We made certain decisions about how overexpression would interact with other aspects of regulation, for example that an overexpressed protein could localize at the SPB without the proteins usually required for localization there. This was inspired by the fact that deletion of *TEM1*, a protein required for Cdc15 localization at the SPB, can be rescued by overexpression of *CDC15*, suggesting that high levels of Cdc15 can localise at the SPB independently of Tem1 (Rock and Amon [2011]). However there are countereexamples; overexpression of *KIN4* is lethal, as it prevents mitotic exit by inhibiting localization of Bfa1 at the SPB, but this lethality can be rescued by deletion of the PP2A subunit Rts1, which controls localization of Kin4 at the SPB (Chan and Amon [2009]). There is no general rule which can account for both of these behaviours, without additional structural information relating to how these proteins bind to the SPB. This is likely to be a general problem when building compartmental logical models.

We found the steady states of the model fit closely with current understanding of the MEN. In the wild type model there is a single steady state for each of the stages of mitotic exit: metaphase, early anaphase (pre-spindle alignment) and late anaphase (post-spindle alignment). In these steady states, the
patterning of proteins on the SPBs matches the known localization patterns of MEN proteins (Figure 5D). Disruption of the asymmetrical distribution of MEN proteins, such as by the bfa1Δ mutation is accurately captured by the model (Figure 5E).

Overall, the model fits the majority (> 80%) of literature phenotypes and the cases where it cannot represent the behaviour of real cells represent the limits of the logical modelling framework.

4.3 Timing of Mitosis

While mutations affecting the function of the FEAR network are not lethal, they reliably cause a delay to exit from mitosis (Stegmeier et al. [2002]). We wanted to know whether our model could reflect this delay. In our model, Cdc15, Nud1 and CDK participate in a negative feedback loop, which is broken by the counteraction of CDK phosphorylation by Cdc14. Therefore, we predicted that loss of Cdc14 activity prior to full MEN activation would delay MEN activation.

Interpretation of timings in logical models can be difficult. With a synchronous update scheme, timings are meaningless while an asynchronous update scheme has, at best, dimensionless pseudotime represented by the number of discrete time steps executed. However, more accurate than either of these is the translation of the model into a continuous time Markov chain applied on the state space of the model (Stoll et al. [2012]), which can then be simulated with the Gillespie algorithm using the MaBoSS package (Stoll et al. [2012, 2017]). In short, this means nodes are updated independently as a random process, occurring at a specified rate, meaning changes in the network state can be assigned continuous timings. Using MaBoSS we simulated wild type and spo12Δ cells in late anaphase (Figure 6A). For these simulations we did not modify the standard rate parameters, as we wish only to compare the mutants to each other. We found that, as expected, the simulated FEAR mutant cells were significantly delayed in exit from mitosis. Intriguingly, the distribution of exit times in the FEAR mutant is not just shifted right, to longer times, but the shape of the distribution is also altered. The distribution of exit times for spo12Δ has a long tail (Figure S3), indicating that in addition to the increased mean, the variance of the distribution is also increased.

We wanted to know whether this effect was detectable in real cells. Previous studies have used bulk measurements to demonstrate the delay caused by FEAR mutants (Stegmeier et al. [2002]), however to measure the distribution of exit times requires single cell measurements. We used a CDC14-CFP mRuby2-TUB1 strain to quantify the length of mitosis in time lapse videos. We defined entry into anaphase as the first frame showing an extended mitotic spindle and completion of mitosis by disassembly of the spindle (Figure 6B&C). We performed five time courses with side-by-side chambers containing SPO12 and spo12Δ strains (Figure S3A). We found that there were some differences in the mean lengths of anaphase in the SPO12 strain between time courses, possibly due to differences in the time cells spent in the chamber. Therefore, we normalized the times in each time course to the mean of the SPO12 cells.
Qualitatively, the experimental distributions of exit times quite closely matched the simulations (Figure 6D, Table - Mitosis Timings.xlsx), with the spo12Δ distribution shifted to the right with a heavier tail as compared to the SPO12 distribution. To quantify this effect, we compared the coefficient of variation (CV), a scale-free measure of variability, of the distributions (Figure 6E). We found that deletion of the FEAR component caused an increase in CV in both simulation and experiment. This suggests that the FEAR network is important not just for a timely exit from mitosis but also for the robustness of the time spent in anaphase.

4.4 Predicting cell-cell variability in checkpoint competence of SPoC mutants

Having established that the model can represent and predict temporal effects we set out to explore how the timing of mitosis can impact the long term viability of cells. The SPoC delays mitosis while the spindle is misaligned and mutations to SPoC components allow cells with misaligned spindle to exit mitosis prematurely. However, there are differences between SPoC mutants; kin4Δ cells with misaligned spindles spend considerably longer in mitosis than bub2Δ cells (Falk et al. [2016a]). SPoC mutants show considerable cell-cell variability in checkpoint competence, with only a fraction of cells exiting mitosis prior to spindle alignment. Precise measurement of the proportion of mutant cells exiting mitosis prior to spindle alignment shows that bub2Δ cells are more likely than kin4Δ cells to exit mitosis prematurely and become multinucleate (Falk et al. [2016a]). We hypothesised that these differences in timing may explain the differences in outcome between these two mutants.

We modified the existing MaBoSS model to allow for the difference in time spent in anaphase for bub2Δ and kin4Δ cells (Model 6). In this model, the wiring has not changed but the rate of Tem1 activation is higher in the presence of Lte1 (Figure 4). This choice was based on the earlier result that Lte1 inhibits the activity of Bub2-Bfa1 towards Tem1. Note that just as before, this does not necessarily indicate that Lte1 acts as a GEF for Tem1 but may act via a different or even indirect mechanism. To dimensionalize the model, we defined three parameters representing the rate of Bfa1 inhibition in the presence (ρ_{fast}) or absence (ρ_{slow}) of Lte1 and the rate of all other variables (ρ) (Table S1). We chose ρ so that the average length of mitosis in a bub2Δ cell with a misaligned spindle is 25 minutes and ρ_{slow} so that it is 70 minutes for a kin4Δ cell (Figure S4A&B). We found that varying ρ_{fast} had minimal effect on the length of mitosis in any of the tested mutants so it was left at 1 (Figure S4C). With these parameters, we could simulate cells with accurate temporal resolution, allowing us to estimate the exit time distributions of both mutant strains (Figure 7A). We define the time taken for a cell to exit mitosis as the random variable, E.

In order to determine whether mitotic exit or spindle alignment occurs first, we require an estimate for the time taken to align the spindle. In order to match the findings of Falk et al. [2016a], we decided
to model kar9Δ osTIR1 dyn1-AID cells (Falk et al. [2016b]). Upon treatment with auxin, these cells become deficient in both of the parallel spindle alignment pathways and so spindle alignment will progress according to the random motion within the cell. As a simplistic model of this process, we consider the spindle to rotate around the centre of the mother compartment, with its angular displacement \( x(t) \) behaving as a Brownian motion (Figure 7B). If the SPB ever passes into the bud neck then we assume the spindle has aligned and cannot become misaligned again. Then, as it does not matter which of the two SPBs eventually enters the bud, we consider \( x(t) \) \( \in (-\frac{\pi}{2}, \frac{\pi}{2}) \), with alignment occurring if \( x \) passes beyond either \( \frac{\pi}{2} - \theta \) or \( -\left( \frac{\pi}{2} - \theta \right) \), where \( \theta \) is half the angular neck width (Figure 7B & S4E). We assume the orientation of the spindle during metaphase is random, so that the initial value is distributed uniformly \( x(0) \sim U(-\frac{\pi}{2}, \frac{\pi}{2}) \). Example trajectories of \( x(t) \) are shown in Figure 7C. We define the random variable, \( A \), to be the alignment time, when \( x(t) \) first crosses \( \pm \left( \frac{\pi}{2} - \theta \right) \). Note that \( P(A = 0) > 0 \), as the distribution of initial values includes regions within the zone of alignment, corresponding to the fact that the spindle may be already aligned to the mother-bud axis upon spindle extension. We performed 10,000 simulations of \( x(t) \) to generate measurements of \( A \). We then used a cubic spline interpolation on the histogram of alignment times to approximate the Probability Distribution Function (PDF) of \( A \). We used a similar approach to approximate the PDF of the time until mitotic exit, \( E \), from the MaBoSS simulation results (Figure 7D).

With PDFs of \( E \) and \( A \) we can analytically deduce the distribution of the difference \( D = E - A \) as a convolution of the two distributions

\[
f_D(t) = \int_0^\infty f_E(t + s)f_A(s)ds,
\]

(Figure 7E). Numerically integrating the area between the x-axis, \( f_D \) and the line \( x = 0 \) yields the probability that \( D < 0 \), which corresponds to the case that mitotic exit occurs prior to spindle alignment, leading to the creation of a multinucleate cell. Note that \( E \), but not \( A \), depends on the specific SPoC mutation and so must be estimated seperately for each mutant. We applied this approach to 10 mutants, where the proportion of successful cell divisions has been determined experimentally by Falk et al. [2016a]. The proportions predicted by our model fit the behaviour measured experimentally (Figure 7F). The only exceptions to this were the reduction in numbers of multinucleate cells caused by the \( spo12\Delta \) mutation in the \( LTE1-8N \) and \( LTE1-8N kin4\Delta \) backgrounds. Our model predicted a modest reduction in the proportion of multinucleate cells, as a result of the delay caused by loss of FEAR function, while Falk et al. [2016a] measured a more significant reduction.

Overall, these findings suggest that the compartmental logical framework is capable of representing the continuous properties of the system, and can distinguish between “strong” and “weak” SPoC mutants.
4.5 Model predictions

A major strength of the compartmental logical framework is the ability to simulate the impact of mutants that affect localization independently of activity. We simulated the forced localization of each of the 10 MEN proteins that localize to the SPB in the model (Figure 8A). Many of these experiments have already been performed, and in these cases the results mainly agree with our simulations (5/6).

Counterintuitively, Bub2 and Bfa1, which are inhibitors of the MEN, promote mitotic exit in cells with misaligned spindles (Scarfone et al. [2015], Gryaznova et al. [2016]), as does Cdc5 (Caydasi et al. [2017]). The ability of the compartmental, logical model to reproduce the behaviour of forced localization of Bub2 and Bfa1 is an improvement over existing compartmental models of the MEN (Caydasi et al. [2012]), which predict the opposite phenotype (Figure S5). Our model predicts that forced localization of Tem1 and Cdc15 promote mitotic exit in all conditions, including in metaphase. The Cdc15 finding is consistent with experimental results (Rock and Amon [2011]). Experiments with a Tem1-Cnm67 fusion protein showed it could promote mitotic exit with a misaligned spindle but not in metaphase (Valerio-Santiago and Monje-Casas [2011]), however it is worth noting that Nud1, rather than Cnm67 is thought to be the scaffold for Tem1 at the SPB (Scarfone and Piatti [2015]). We decided to test whether Tem1 could initiate mitotic exit if forced to interact with Nud1 using the GFP-Binding Protein (GBP) (Rothbauer et al. [2007]). We expressed NUD1-GBP from the reduced strength GALS promoter in either wild type, TEM1-YFP or CDC15-YFP strains. We found that recruitment of Cdc15 but not Tem1 to the SPB could promote mitotic exit in cells arrested in metaphase (Figure S6A, Supplementary File 8). We also found that recruitment of either Tem1 or Cdc15 was lethal to the cell (Figure S6B). Therefore, there must be additional levels of regulation of Tem1 at the SPB that are not currently represented in the model.

Kin4 is predicted to prevent mitotic exit when forced to localise at the SPB. The experimental evidence for this mutation is conflicted, with a Kin4-Spc72 fusion causing a delay to mitotic exit (Maekawa et al. [2007]), while a symmetrically localising mutant caused no delay (Chan and Amon [2010]). Kin4 was found to cause a significant growth defect when bound to Spc72 in genome-wide synthetic physical interaction screens (Howell et al. [2019]). Dbf2, Mob1 and Cdc14 are all predicted to be insufficient to alter MEN signalling at the SPB. Of these only Mob1 has been tested, it was found that a Mob1-Nud1 fusion was in fact lethal (Rock et al. [2013]), presumably because it prevented movement of Mob1-Dbf2 into the nucleus, an effect not captured by this model. CDK is predicted to behave like Kin4, preventing mitotic exit when forced to localise to the SPB and further this effect is predicted to be rescued by bfa1Δ (Figure 8B). A study found that forced localization of Clb2 to the SPB delayed spindle disassembly (Yang et al. [2013]), however no experiments forcing CDK to the SPB have been performed.

We constructed strains expressing NUD1-GFP from the endogenous promoter and bearing plasmids expressing either GBP or a fusion CLB2-CDC28-GBP protein from the MET3 promoter. We tuned the
expression of fusion protein by addition of 10μM methionine, to prevent high levels of CDK overexpression (Mao et al. [2002]). We found that forcible recruitment of CDK to the SPB caused a growth defect (Figure 8C) and that this growth defect was rescued by bfa1Δ. Recruiting CDK to the SPB caused cells to arrest in late anaphase, with a large bud and an extended spindle (Figure S6C) and this phenotype was rescued by bfa1Δ (Figure S6D, Supplementary File 9). This indicates a failure to exit from mitosis, as our model predicts.

Forcible localization of proteins to both SPBs has been used as a tool to explore the impact of localization for many years however forcing proteins to localize to a single SPB has not been explored in the same detail. An optogenetic binding system has been used to target Clb2 to a single SPB (Yang et al. [2013]) however the downstream impact on MEN signalling was not fully investigated. Our compartmental logical model is capable of making predictions of the outcomes of such experiments. Our model predicts that targeting Cdc15 to either of the SPBs would be sufficient to drive mitotic exit (Figure 8D). This suggests that MEN signalling could occur at the mSPB, if Cdc15 was present there. On the other hand, the inhibitory effect of CDK is predicted to occur only when CDK is targeted to the dSPB, with forcible localization of CDK at the mSPB having no functional effect (Figure 8D).

5 Discussion

In this article, we have presented a compartmental, logical model of the control of mitotic exit in yeast. This novel modelling formalism brings together the spatial resolution of compartmental ODE models and the simplicity and scalability of logical models. This model can act as both a representation of our knowledge of the MEN and a tool to guide experimental design; in this article we have done both. The choice of the logical formalism comes with inevitable limitations, as reducing the complexities of protein dynamics to discrete levels of activity is a significant simplification. In some cases, such as the treatment of Kin4 regulation of Bub2-Bfa1, we were able to simplify a quantitative mechanism, in this case a change of turnover rate, into a simpler mechanism whereby Kin4 simply keeps Bub2-Bfa1 off the SPB. In other cases, the model has not been able to represent effects such as low level localization of MEN proteins in metaphase (Bardin et al. [2000], Pereira et al. [2000]) or symmetric localization of MEN proteins late in anaphase (Campbell et al. [2020]). The implicit representation of complexes in this model also represents a further limitation. It is possible these limitations could be overcome, for example by addition of extra levels for these nodes, however it seems likely that some mechanisms will never be fully captured by logical rules. We argue that despite these limitations, the simplicity of the formalism make it a useful tool to explore networks like the MEN.

The steps taken to optimize and refine the model provide some insight into key aspects of MEN regulation. We included two levels of Bub2-Bfa1 and Tem1 activation in order to accurately model the
effect of bub2Δ or bfa1Δ mutations. The necessity of this step shows the importance of Bub2-Bfa1 to tune the strength of MEN response throughout mitosis. In order for kin4Δspo12Δ cells to maintain a SPoC, we required two parallel Bub2-Bfa1 regulation pathways (Figure 9). This mechanism was first proposed by Falk et al. [2016a], but the molecular details are still unclear. Model 6 proposed that Lte1 activity is also important for the timing of MEN signalling in the presence of Bub2-Bfa1. Lte1 has long been known to contain a GEF-like domain, however no GEF activity towards Tem1 could be detected in vitro (Geymonat et al. [2009]). Therefore either Lte1’s Kin4-independent activity towards Bub2-Bfa1 acts through an as yet unknown intermediate protein or Lte1’s GEF activity depends on factors not included in the reactions of Geymonat et al. [2009], for example scaffolding by SPB components. While experiments show the lte1Δkin4Δspo12Δ triple mutant struggles to exit mitosis, it is still able to do so with low efficiency (Caydasi et al. [2017]), demonstrating that other bud-localised proteins, such as Ste20, can promote MEN activity. Indeed, this model uses a coarse-grained representation of polarity proteins, and the model could be expanded to represent known interactions of the MEN with Ste20, Kel1&2, Cdc24 and Cdc42 (Höfken and Schiebel [2002]). The lte1Δkin4Δspo12Δ mutation prevents exit from mitosis in Model 4, as Ste20 is not currently represented in the model, although this could be implemented in future versions. Finally, in order to fit the phenotype of CDC15-7A MOB1-2A cells, we introduced an ASC responsible for controlling Cdc15 localization in the absence of Tem1 and CDK. Integrating the findings of Rock and Amon [2011] and Botchkarev et al. [2017], we propose that the movement of Cdc5 across the nuclear membrane to the cytoplasmic face of the SPB in anaphase is the signal represented by the ASC (Figure 9). Botchkarev et al. [2017] propose that the translocation of Cdc5 is controlled by the FEAR network, and this could be tested in future models. Our model predicted that the FEAR network is important not just for timely mitotic exit, but also to even out variation in the length of mitosis (Figure 9). This finding was validated by experimental measurements of the length of anaphase in wild type and FEAR mutant cells. While the FEAR mutants have long been known to interact genetically with MEN mutants, the purpose of the network, beyond speeding up anaphase, has been difficult to understand. Our solution is that FEAR is primarily a mechanism to limit the variability of the length of mitosis. In our model, it is the activity of FEAR towards Cdc15 which determines anaphase variability however other aspects of regulation may play a role. For example, Cdc14 reverses CDK phosphorylation of securin, creating a positive feedback loop in the FEAR and sharpening the metaphase-anaphase transition (Holt et al. [2008]). Future versions of the model could be updated to include this. It is interesting to note that single cell measurements of cell cycle stages in human cells show they follow an Erlang distribution, which represents the time taken by k independent Poisson processes of rate λ (Chao et al. [2019], Gelens and Santos [2019]). There is a direct parallel here to the continuous-time Markov chain used by MaBoSS, in which the update times for each node are independent Poisson processes. This suggests that this implementation of the logical
modelling framework will be an effective tool to model the length of cell cycle stages.

We have proposed a link between the speed of MEN activation and the strength of SPoC mutants, which is capable of reproducing the behaviour of a number of mutants. Furthermore, we found that after fitting rate parameters to the logical model, we were able to fit the percentage of multinucleate cells in both \( \text{kin}4\Delta \) and \( \text{bub}2\Delta \) by fitting a single parameter, \( \sigma \). This suggests the model is not overfitted to the data. This demonstrates how the qualitative logical approach can be easily adapted to make quantitative predictions. Our approach, based on the difference in timing between checkpoint satisfaction and signalling, is likely to apply more broadly to the study of other checkpoints which have mutants of varying strengths.

Our model correctly predicted the phenotype caused by forcing most MEN proteins to localize at the SPBs. This demonstrates that the compartmental logical framework is versatile enough to make predictions about the impact of protein mislocalization. Protein mislocalization is a powerful tool to probe protein function (Ólafsson and Thorpe [2015]) and compartmental logical models may aid in guiding design of these experiments. Synthetic physical interaction screens with the SPB found an enrichment for MEN proteins among proteins which cause a growth defect when forced to interact with Nud1 (Howell et al. [2019]), as well as identifying mitotic regulators such as Glc7 (PP1) and Tpd3 (PP2A).

The model incorrectly predicts that forcing Tem1 to the SPB can initiate mitotic exit in metaphase; as demonstrated by the experiments of both Valerio-Santiago and Monje-Casas [2011] and ourselves. The \( \text{bub}2\Delta \) or \( \text{bfa}1\Delta \) mutations cause mitotic exit to occur in metaphase by promoting premature Tem1 loading, so exploring why this mutation but not \( \text{TEM1-SPB} \) can have this effect will be an interesting direction for further experimentation. It is also interesting to note that some mutations that lose control of mitotic exit have no impact on viability (such as \( \text{bub}2\Delta \)), while perturbations such as Cdc15-SPB are lethal. While many experiments have been performed forcing protein localization at both SPBs, none have explored the impact of forcing MEN proteins to a single SPB. Our model predicts that the MEN could be activated in metaphase or in cells with misaligned spindles if Cdc15 is targeted to either SPB. This could result in the reversal of the usual asymmetry, with MEN activity occurring at the mSPB rather than the dSPB. On the other hand, our model predicts that the inhibitory effect of forced CDK localization would occur only when CDK is targeted to the dSPB, with no effect resulting from forced localization at the mSPB. It will be an important test of the model to conduct these experiments, which could be performed with an optogenetic binding system such as Yang et al. [2013].

Our model of the MEN can represent \( \sim 80\% \) of the genetic phenotypes we tested in the validation stage, for a total of 161/195 phenotypes matching experiments when combined with the FEAR network training set. These phenotypes include deletions, temperature-sensitive alleles, overexpressions and most importantly for this study, localization mutants. This success demonstrates how the logical modelling framework is capable of capturing many of the essential aspects of MEN regulation. However, there are
still some phenotypes the model cannot predict, and in particular the model struggles to represent over-
expression and temperature-sensitive alleles. Overexpression is a complex genetic perturbation (Moriya
[2015]) and as we demonstrate with the examples of $GAL1p$-$CDC15$ $tem1\Delta$ and $GAL1p$-$KIN4$ $rts1\Delta$ a
single set of rules for how overexpression interact with other aspects of regulation is not possible. Look-
ing forward, we must either accept the low level of inaccuracies caused by blanket treatments or find
ways to integrate further quantitative details about how proteins interact in order to make more precise
predictions about how overexpression may interact with other mutations. Temperature sensitivity is also
a difficult perturbation to model, with the model treating such mutants as identical to full deletions.
Dealing with this limitation will be particularly important if genome-scale models are to be benchmarked
against systematic genetic interaction datasets such as Costanzo et al. [2016]. Existing approaches to
prediction of genetic interactions from logical models still do not allow for partial loss of function mu-
tations, for example Calzone et al. [2015]. If a methodology to understand the impact of partial loss of
function mutations could be developed, this would open the doors to the use of logical models to predict
phenotype from genotype at the genome scale, with important applications to personalised medicine.

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5.2 Author Contributions

R.S.M.H., P.H.T. and A.C-N. contributed to conception of the work. R.S.M.H. contributed to coding
and development of the computational model. R.S.M.H. and C.K. contributed to data collection and
analysis. R.S.M.H. drafted the article; all authors contributed to critical revision and gave final approval
for publication.

5.3 Competing Interests

The authors declare no competing financial interests.
Figure 1: A simplified graphical description of the MEN and FEAR pathways. A detailed description of both networks can be found in the Supplementary Information.
Figure 2: The compartmental logical modelling framework and the MEN. A: The compartments present in the model. B: Illustration showing how a simple biochemical motif can be interpreted as a set of logical rules, shown as a truth table. C: Schematic showing how a logical network can be expanded across multiple compartments, with additional rules to describe the regulation of localization.
Figure 3: Refinement of the MEN model based on mutants that can release Cdc14 in metaphase. All simulations are performed using the random asynchronous update scheme, 100 cells were simulated for each mutant starting from realistic initial conditions. In the original model, the \textit{CDC15-7A} mutant has an intact SPoC, in contradiction of the experimental evidence. Introducing regulation of the Cdc15\textsubscript{High} SPB localization node by CDK fixes this issue (Model 1) however this model cannot fit the behaviour of the \textit{CDC15-7A MOB1-2A} double mutant. This double mutant can exit mitosis in metaphase but only when the spindle aligns and an SPB enters the bud (Campbell et al. \cite{2019}). The inclusion of an Anaphase-Specific Component (ASC) that limits Cdc15 loading in metaphase resolves this problem (Model 2). Deletion of either component of the Bub2-Bfa1 GAP complex also permits exit from mitosis only when the spindle aligns and an SPB enters the bud (Campbell et al. \cite{2019}). The inclusion of two levels of Bub2, Bfa1 and Tem1 activity (Model 3) is sufficient to represent this effect. All simulation data can be found in Supplementary File 5.
Figure 4: Refinement of the MEN model based on the phenotype of $\text{kin4}\Delta\text{spo12}\Delta$ cells. All simulations are performed using the random asynchronous update scheme, 100 cells were simulated for each mutant starting from realistic initial conditions. In Model 3, the double mutant $\text{kin4}\Delta\text{spo12}\Delta$ did not have a SPoC, in disagreement with experimental evidence (Falk et al. [2016a]). By introducing an additional level of regulation of Bfa1 by Lte1, this issue was resolved in Model 4. This change also allowed for identification of the ASC with Cdc5, while maintaining the correct behaviour of related phenotypes, such as CDC15-7A MOB1-2A, $\text{kin4}\Delta$ and $\text{cdc5-1}$. All simulation data can be found in Supplementary File 5.
Figure 5: Validation of Model 5 against literature phenotypes. A: The model correctly predicted 81% of the 140 tested literature phenotypes (Supplementary File 6). B: The model often failed at predicting the phenotype of cells with a genotype that mixes overexpression with other mutations, such as the rescue of the temperature sensitive alleles cdc15-2 and dbf2-2 by overexpression of CDC5. C: The model predicts that overexpression of CDC5 cannot rescue the mob1Δ mutation. D: Spot test confirming the model prediction that overexpression of CDC5 can not rescue full deletion of MOB1. A mob1Δ strain kept alive by provision of a CEN-MOB1 plasmid with uracil selection, was transformed with either a 2µm plasmid bearing MOB1 or CDC5 or an empty plasmid. The CEN-MOB1 plasmid was counter-selected by addition of 5FOA, showing that moderate overexpression of Cdc5 is not sufficient for rescue of the mob1Δ phenotype. E: The localization state of MEN proteins on the SPBs in the three physiological stages of mitotic exit in the model. Steady states determined from synchronous update scheme. F: Comparison of (a)symmetry of SPBs in the steady states of wild type and bfa1Δ cells. All simulation data can be found in Supplementary File 5.
Figure 6: The role of FEAR in regulating anaphase length. A: We simulated 10,000 SPO12 and spo12Δ cells and the length of anaphase (time from model initiation until mitotic exit) was calculated, and was normalized to the mean of the wild type cells. B: Schematic showing the key cell cycle events used to calculate the length of time spent in anaphase. C: Time course showing mRuby2-Tub1 fluorescence in a representative cell during exit from mitosis. Images were taken at 2 minute intervals and used to determine the length of anaphase. The image at 0 minutes shows the final frame where the cell has an un-extended spindle and spindle disassembly after 18 minutes. D: Distribution of anaphase lengths in SPO12 and spo12Δ cells. 5 time course were performed, each with 3 fields of view per strain, (SPO12 n = 281, spo12Δ n = 223). Due to differences in mean exit times between time courses, exit times from each time course were normalized to the mean exit time of SPO12 cells in that time course. E: The coefficient of variation of exit times for SPO12 and spo12Δ cells in simulation and experiment. Raw data can be found in Supplementary File 7.
Figure 7: Use of the parameterised model to predict and explore cell-cell variability in SPoC mutants. A: Simulated exit times of \( \textit{bub2}\Delta \) and \( \textit{kin4}\Delta \) cells with misaligned spindles, from 10,000 runs of the model. B: Schematic of a \( \textit{kar9}\Delta \textit{osTIR1 dyn1-AID} \) cell, showing the spindle angle \( x(t) \) and the half-angular neck width, \( \theta \). C: Simulations of \( x(t) \), the spindle angle starting from uniformly distributed initial conditions and varying as a Brownian motion. The time until alignment \( A_i \) is indicated for each simulation. \( A_1 = 0 \) as in this case the initial condition of the simulation is within the bud neck (\( x(0) > \frac{\pi}{2} - \theta \)), corresponding to the scenario where the spindle is aligned at the point of extension. \( A_2 \) and \( A_3 \) can be measured as the point where \( x(t) \) crosses either of the boundaries, as it is not important which SPB enters the bud. The final two simulations do not achieve alignment during the 60 minutes simulated so \( A_4, A_5 > 60 \). D: The distribution of exit times, \( E \), for a simulated \( \textit{bub2}\Delta \) mutant and the distribution of alignment times, \( A \), for a simulated \( \textit{kar9}\Delta \textit{osTIR1 dyn1-AID} \) cell. These distributions were inferred from cubic spline interpolation of histograms generated from 10,000 runs of the model or 10,000 Brownian motion simulations respectively. E: Distribution of the difference between exit time and alignment time, \( D \), for the simulated \( \textit{bub2}\Δ \textit{kar9}\Δ \textit{osTIR1 dyn1-AID} \). The area between the x-axis, the curve and \( x = 0 \) gives the predicted probability of a given cell exiting mitosis before spindle alignment occurs, giving rise to a multinucleate cell. F: Predicted proportions of multinucleate cells for various genetic backgrounds. Dotted lines show the measured proportions of multinucleate cells in Falk et al. [2016a].
Figure 8: Forced localization phenotypes. A: Predicted phenotype of cells where each of the SPB-localized proteins in the model are forced to localize to the SPB. A question mark (?) indicates a phenotype that has not been experimentally verified in the literature. A star (*) is used to indicate that the MOB1-SPB phenotype differs from literature accounts due to factors beyond the scope of the model. B: Predicted rescue of the CDK-SPB phenotype by bfa1Δ. C: Spot tests showing growth defect of Nud1-GFP cells expressing a fusion Clb2-CDK-GBP protein from the MET3 promoter and rescue of this defect by bfa1Δ. Activity of the MET3p promoter was tuned by addition of 10µM methionine to media. D: Predicted phenotype of cells where Cdc15 or CDK are forced to either the mSPB or dSPB.

All simulation data can be found in Supplementary File 5.
Figure 9: Model of the MEN including the developments contributed in this manuscript. A: Lte1 regulates Bub2-Bfa1 via two pathways, only one of which is dependent on Kin4. B: Cdc5 is required for recruitment of Cdc15 to the SPB in the absence of Tem1 or CDK regulation. C: FEAR breaks a Cdc15-Nud1-CDK negative feedback loop, leading to deterministic timing of mitosis.
Table S1: Parameters used to simulate SPoC competence.

| Parameter | Definition | Value |
|-----------|------------|-------|
| $\rho$    | Standard rate of reactions in Model 6 | 0.84  |
| $\rho_{\text{slow}}$ | Rate of Tem1 activation in absence of Lte1 | 0.012 |
| $\rho_{\text{fast}}$ | Rate of Tem1 activation in presence of Lte1 | 1     |
| $\sigma$  | Rate constant of spindle alignment | 0.14  |
| $\theta$  | Half-angular bud width | 0.3   |

Figure S1: A: Activity and localization networks underlying the compartmental model. B: Schematic showing how logic gates are expanded to include overexpression (OE) nodes and the corresponding logic gate.

| Key          | Activity network | Localization network | Models 3-4 | Models 4+ | Model 5 |
|--------------|------------------|----------------------|------------|----------|--------|
|              | -                | -                    |            |          |        |
| A B A\_OE C | 0 0 0 0 1        | 0 1 0 0 0            | 1 0 0 0 1  | 1 1 0 0 1 | - 0 1 0 1 |
|              | -                | -                    | 0 1 0 1    | 1 1 1 1 1 | -      |
Figure S2: Identifying the ASC as Cdc5 in Model 3 (Model 3a) leads to incorrect behaviour of CDC15-7A MOB1-2A. Model 3 requires an additional pathway linking Lte1 to Tem1. In Model 4 Lte1 directly inhibits Bfa1 activity, leading to correct prediction of the phenotype of GAL1p – KIN4 cells. A version where Lte1 directly activates Tem1 (Model 4a) predicts behaviour of GAL1p – KIN4 incorrectly. All simulation data can be found in Supplementary File 5.
Figure S3: A: Raw measurements of anaphase length, grouped by strain and time course. Box plots show the median and the upper and lower quartiles, whiskers show 1.5 times the inter-quartile range or the closest measurement, whichever is closest. B: Log density distribution of simulated exit times for SPO12 and spo12Δ cells. The spo12Δ distribution show a power law tail, while the SPO12 distribution does not. C: Log density distribution of experimentally observed exit times for SPO12 and spo12Δ cells. Both distributions show a power law tail, however the gradient of the spo12Δ distribution is shallower.
Figure S4: Parameter selection for the dimensional model (Model 6). A: The basic rate, $\rho$, was chosen so that the mean of the exit time distribution of $bub2\Delta$ is 25. We simulated 10,000 anaphase cells with misaligned spindles for 40 values of $\rho$ between 0.6 and 1.0 and calculated the mean exit time. The closest to the target value ($\rho = 0.84$) was selected. B: The slow rate of Bfa1 inhibition, $\rho_{\text{slow}}$, was chosen so that the mean of the exit time distribution of $\text{kin4}\Delta$ is 70. We simulated 10,000 anaphase cells with misaligned spindles for 18 values of $\rho_{\text{slow}}$ between 0.004 and 0.018 and calculated the mean exit time. The closest to the target value ($\rho_{\text{slow}} = 0.012$) was selected. C: We tried varying the fast rate of Bfa1 inhibition, $\rho_{\text{fast}}$, over 2 orders of magnitude but found it had little effect on the length of mitosis in either mutant, so it was left at $\rho_{\text{fast}} = 1$. Mean exit times were derived from simulations of 10,000 anaphase cells with misaligned spindles. D: The parameter, $\sigma$, representing the rate of spindle alignment, was chosen to match both the measured proportions of multinucleate cell formation in $bub2\Delta$ ($\sim 0.5$) and $\text{kin4}\Delta$ ($\sim 0.25$). We tested 6 values of $\sigma$ between 0.11 and 0.16. Fortunately, the value $\sigma = 0.14$ fits both proportions closely. Mean exit times were derived from simulations of 10,000 anaphase cells with misaligned spindles. E: Measurement of the half-angular bud width, $\theta$, from a microscope image of a large-budded wild type cell. Based on this measurement we use a value of $\theta = 0.3$. 
Figure S5: Simulations of the model of Caydasi et al. [2012]. In this model, activation of the MEN is signalled by the number of Tem1-GTP molecules exceeding 65 (the MEN threshold). Simulations were performed using the same parameters as Caydasi et al. [2012], with custom initial conditions matching the pre-alignment steady states of the model.
Figure S6: A: Recruiting Cdc15 but not Tem1 to the SPB promotes mitotic exit in metaphase. Wild type, TEM1-YFP and CDC15-YFP cells expressing NUD1-GFP from a plasmid were synchronized with alpha factor and then arrested in metaphase withnocodazole. After 3 hours the number of single and multi-budded cells was counted. Error bars represent 95% confidence intervals calculated with the Clopper-Pearson method. P-values were calculated using the two-tailed Fisher’s exact test. B: Forced interaction of both Tem1 and Cdc15 with Nud1 is lethal. C: Representative images of NUD1-GFP MET3p-CLB2-CDC28-GBP-RFP cells, grown in media containing 10µM methionine. We placed cells into 4 categories: G1, pre-anaphase spindle (S or early M cells with 2 SPBs less than 3µm apart), anaphase (SPBs over 3µm apart) and abnormal cells (aberrant SPB or bud number). D: Quantification of the percentage of cells in each category. NUD1-GFP MET3p-CLB2-CDC28-GBP-RFP cells showed a high proportion of anaphase cells, which could be rescued by repression of the MET3 promoter by addition of methionine or by the bfa1Δ mutation. MET3p activity was tuned by addition of 0.01mM methionine (+) or 2mM methionine (-). P-values calculated using the two-tailed Fisher’s exact test.
| Plasmid Name | Genotype | Type | Selection | Source               |
|--------------|----------|------|-----------|----------------------|
| pX29         |          | CEN  | Leu/Amp   | Reid et al. [2011]   |
| pX78         | CDC5     | 2µm  | Leu/Amp   | Caydasi et al. [2017]|
| pX79         | MOB1     | 2µm  | Leu/Amp   | Caydasi et al. [2017]|
| pX80         | MOB1     | CEN  | Ura/Amp   | Caydasi et al. [2017]|
| pHT790       | MET3p-GBP-RFP | CEN | Leu/Met/Amp | this study          |
| pHT795       | MET3p-CLB2-CDC28-GBP-RFP | CEN | Leu/Met/Amp | this study          |

Table S2: Plasmids used in this study

| Strain name | Genotype | Source               |
|-------------|----------|----------------------|
| CKY329-3    | mob1Δ::klTRP1 | Caydasi et al. [2017] |
| T744        | CDC14-CFP::shHPH HIS3p-mRuby2-TUB1+3’UTR::URA3 | this study          |
| pT747       | CDC14-CFP::shHPH HIS3p-mRuby2-TUB1+3’UTR::URA3 spo12Δ::KAN | this study          |
| E438        | -        | Brachmann et al. [1998] |
| T724        | CDC15-YFP::KAN | this study          |
| T725        | TEM1-YFP | this study          |
| Nud1-GFP    | Nud1-GFP::HIS3 | Huh et al. [2003] |
| pHT795      | Nud1-GFP::HIS3 bfa1Δ::KAN | this study          |

Table S3: Strains used in this study
7 Supplementary Information

7.1 Abbreviations

• APC - Anaphase Promoting Complex
• ASC - Anaphase Specific Component
• CDK - Cyclin-Dependent Kinase
• FEAR - CdcFourteen Early Anaphase Release
• GBP - GFP-binding protein
• MEN - Mitotic Exit Network
• ODE - Ordinary Differential Equations
• PDF - Probability Distribution Function
• SAC - Spindle Assembly Checkpoint
• SIN - Septation Initiation Network
• SPB - Spindle Pole Body
• SPoC - Spindle Position Checkpoint

7.2 The FEAR Network

The FEAR network is centred on phosphorylation of Net1, which leads to disassociation of Cdc14 from Net1 (Rock and Amon [2009]) (Figure 1). Net1 is phosphorylated by CDK, Cdc5 and Mob1-Dbf2 and some of this phosphorylation is reversed by PP2A-Cdc55 (Shou et al. [2002], Azzam et al. [2004], Ptacek et al. [2005], Queralt et al. [2006]). The FEAR signal is initiated by release of separase (Esp1) caused by destruction of securin (Pds1) by APC-Cdc20 upon release of the SAC. Esp1 functions in a complex with Slk19 and in conjunction with Zds1/2 to downregulate PP2A-Cdc55 and exclude it from the nucleus (Queralt and Uhlmann [2008], Rossio and Yoshida [2011]). This shifts the kinase/phosphatase balance in the nucleus to allow the hyper-phosphorylation of Net1 by CDK and Cdc5 that is required for FEAR. Further FEAR components are the nucleolar proteins Spo12 and Fob1, which participate through a poorly understood mechanism (Stegmeier et al. [2004], Tomson et al. [2009]). There is some discussion whether Cdc14 is limited to the nucleus in early anaphase (Yellman and Roeder [2015]). Certainly it is not detectable by fluorescence microscopy outside of the nucleus at this stage, however the genetic interactions between FEAR and MEN components (Jaspersen et al. [1998]) suggest that it may be present in the cytoplasm at low levels, where it dephosphorylates cytoplasmic MEN components.
7.3 The MEN

Activation of the MEN is controlled by the SPoC which targets the Tem1 GTPase. Tem1 is regulated by the Bub2-Bfa1 complex, which acts as a GTPase-Activating Protein (GAP), forcing Tem1 into its inactive GDP-bound state (Pereira et al. [2000]). Bub2-Bfa1 localises asymmetrically to the Spindle Pole Bodies (SPBs), with a preference for the SPB that is closest to the bud (the daughter-bound SPB or dSPB, as opposed to the mother-bound SPB or mSPB). Tem1 also localises to the SPBs, primarily through interaction with Bub2-Bfa1, although, to a lesser extent, independently (Pereira et al. [2002], Caydasi et al. [2012]). Bub2-Bfa1’s GAP activity can be inhibited by phosphorylation of Bfa1 by Cdc5 (Geymonat et al. [2003]), which also resides at the SPBs (Botchkarev and Haber [2017]). Bub2-Bfa1 localization is regulated by Kin4, which kinase protects Bfa1 by disrupting its stable localization at the SPB, keeping it away from Cdc5 (Caydasi and Pereira [2009]). This means that in the mother compartment, where Kin4 resides, Bfa1 is kept active (Maekawa et al. [2007]). When the dSPB enters the bud, Bfa1 is no longer protected by Kin4, allowing Cdc5 phosphorylation to occur. Kin4 itself is excluded from the bud compartment by the bud-localised protein Lte1 (Bertazzi et al. [2011], Falk et al. [2011]). Aside from regulation of Kin4, Lte1 has additional MEN-promoting activity, possibly acting through Bfa1 (Caydasi et al. [2017]). Lte1 has homology with other GEFs, and it was this activity which was thought to oppose the GAP activity of Bub2-Bfa1 towards Tem1 (Bardin and Amon [2001]), however no GEF activity towards Tem1 was detected in vitro (Geymonat et al. [2009]). Upon entry of the dSPB into the bud, the Bub2-Bfa1 complex becomes inactive, allowing Tem1 to recruit the kinase Cdc15 to the SPB (Rock and Amon [2011]), which in turn recruits the Dbf2-Mob1 complex (Rock et al. [2013]). There Cdc15 phosphorylates Dbf2 (Mah et al. [2001]), activating the Dbf2-Mob1 complex which in turn leads to the full release of Cdc14. The exact details of the mechanism by which Mob1-Dbf2 promotes mitotic exit are not yet understood, however it is known that the complex enters the nucleus (Stoepel et al. [2005]), phosphorylates Net1 (Ptacek et al. [2005]) and phosphorylates Cdc14 near to its NLS, allowing Cdc14 to leave the nucleus (Mohl et al. [2009]).

Most of the MEN proteins localise to the SPB through interaction with the MEN scaffold, Nud1, however Kin4 interacts with Spc72 (Gryaznova et al. [2016]). To an extent, Bub2-Bfa1 may be considered an additional scaffold, as Tem1 localization is partially dependent on Bub2-Bfa1 (Caydasi et al. [2012]), as is the localization of Cdc14 (Pereira et al. [2002]). Together with Cnm67, Nud1 and Spc72 form the outer plaque of the SPB, situated on the cytoplasmic face of the structure (Fu et al. [2015]).

As FEAR release is not essential for MEN activity it has been difficult to pinpoint exactly how it contributes to mitotic exit. Cdc15 and Mob1 kinase activity are both inhibited by CDK phosphorylation (Jaspersen and Morgan [2000], König et al. [2010]), limiting their activity in metaphase, and phosphorylation of Bfa1 is important for SPoC function (Caydasi et al. [2017]). Patterns of Cdc15 and CDK localization at SPBs suggest that their SPB localization is mutually exclusive, and so FEAR activity
may be required in anaphase to allow Cdc15 to interact with the SPB (König et al. [2010]). Furthermore, CDK and Cdc14 have been shown to contribute to regulation of Bfa1 by Lte1 in the absence of Kin4 (Caydasi et al. [2017]).

7.4 Scope of the model

Our proposed model aims to represent the above aspects of regulation of Cdc14 localization from metaphase to telophase. The roles of the MEN in cytokinesis and spindle positioning are considered outside of this scope, as is the execution of mitotic exit. In certain cases we have found differing phenotypes published for similar mutants, see for example length of mitosis in FEAR mutants (see Stegmeier et al. [2002] and Yellman and Roeder [2015]) and the role of Bmh1 in the SPoC (see Caydasi et al. [2014] and Falk et al. [2016a]). In these cases we have chosen a specific account to form the basis of the model. Some mutants, such as those of LTE1 (Low Temperature Essential), show differing phenotype at low temperature (Shirayama et al. [1994a]), so our model aims to represent the cell cycle at 30°C. The impact of loss of SPoC function on cell viability is not generally detectable in yeast in lab conditions, as the efficiency of spindle alignment is so high that it will generally occur prior to cytokinesis regardless of whether it is monitored. There are two independent spindle orientation pathways, based on either Kar9 or Dyn1, loss of either of these proteins leads to a significant delay in spindle alignment and so these mutants are used to detect SPoC defects (Scarfone and Piatti [2015]). In our model we assume that for any SPoC phenotypes, we model a cell defective in one of these pathways.

7.5 Supplementary Materials

7.5.1 Supplementary Files

1. PKN used to train FEAR network model.
2. Literature phenotypes used to train FEAR network model.
3. List of edges included in Model 5, with justification for each edge.
4. Initial conditions used to simulate logical model.
5. Data from all simulations shown in Figures 3, 4, 5, 8 and S2.
6. Model validation data, including mutant, literature phenotype, citation and simulation phenotype.
7. Anaphase length measurements.
8. Data from rebudding after Nocodazole arrest experiment.
9. Quantitative microscopy data from CLB2-CDC28-GBP NUD1-GFP strains.
7.5.2 Supplementary Video

Time lapse video showing anaphase in two \textit{spo12}\Delta cells, arrows indicate the time and location of spindle breakage. Spindles are visualized with an mRuby2-Tub1 marker (left) and cell morphology is imaged using bright-field microscopy (right). The time between the first frame of spindle extension and the first frame of spindle disassembly (arrows) is used to determine anaphase length.
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