**In silico** study of kinetochore control, amplification, and inhibition effects in MCC assembly

Bashar Ibrahim\textsuperscript{a,b}, Eberhard Schmitt\textsuperscript{c}, Peter Dittrich\textsuperscript{a,b,}\textsuperscript{*}, Stephan Diekmann\textsuperscript{c}

\textsuperscript{a} Bio Systems Analysis Group, Institute of Computer Science, Friedrich-Schiller-University Jena, Germany  
\textsuperscript{b} Jena Centre for Bioinformatics (JCB), Jena, Germany  
\textsuperscript{c} Leibniz Institute for Age Research (FLI), Jena, Germany

**Abstract**

Eukaryotic cells rely on a surveillance mechanism, the “Spindle Assembly Checkpoint” (MSAC) in order to ensure accurate chromosome segregation by preventing anaphase initiation until all chromosomes are correctly attached to the mitotic spindle. In different organisms, a mitotic checkpoint complex (MCC) composed of Mad2, Bub3, BubR1/Mad3, and Cdc20 inhibits the anaphase promoting complex (APC/C) to initiate promotion into anaphase. The mechanism of MCC formation and its regulation by the kinetochore are unclear. Here, we constructed dynamical models of MCC formation involving different kinetochore control mechanisms including amplification as well as inhibition effects, and analysed their quantitative properties. In particular, in this system, fast and stable metaphase to anaphase transition can only be triggered when the kinetochore controls the Bub3:BubR1-related reactions; signal amplification and inhibition play a subordinate role. Furthermore, when introducing experimentally determined parameter values into the models analysed here, we found that effective MCC formation is not combined with complete Cdc20 sequestering. Instead, the MCC might bind and completely block the APC/C. The MSAC might function by an MCC:APC/C complex rearrangement.

© 2008 Elsevier Ireland Ltd. All rights reserved.

**1. Introduction**

Cell division requires well controlled chromosome segregation into the two daughter cells. In mitosis, the “Spindle Assembly Checkpoint” (MSAC) (Minshull et al., 1994) ensures accurate segregation by delaying anaphase initiation until all chromosomes are attached to microtubuli and aligned at the metaphase plate. Incorrect chromosome segregation may lead to aneuploidy (Kim and Kao, 2005; Steuerwald, 2005) or cancer (Compton, 2006; Gupta et al., 2003) (for reviews see Musacchio and Salmon, 2007; Kops et al., 2005; Zhu et al., 2005; Taylor et al., 2004; Musacchio and Hardwick, 2002).

To prevent anaphase onset before all kinetochores are connected to microtubuli and microtubuli have come under tension, the MSAC inhibits the APC/C (“Anaphase Promoting Complex/Cyclosome”): an E3 ubiquitin ligase Yu, 2002; Sudakin et al., 2001; Hwang et al., 1998), which becomes active by forming the APC/C\textsubscript{Cdc20} complex with its co-activator Cdc20 (“Cell division cycle 20” homologue Hwang et al., 1998; Jeganathan and van Deursen, 2006). The exact molecular mechanisms are still unclear. Two processes contributing to this APC/C inhibition have been identified: sequestering of Cdc20 and direct inhibition of the APC/C by the MCC (“Mitotic Checkpoint Complex”). During metaphase, the concentration of free Cdc20 is low. Cdc20 can be bound to Mad2 to form a Mad2:Cdc20 complex. In addition, Cdc20 can form the MCC together with Mad2, BubR1, and Bub3. The two proteins Mad2 (Fang, 2002; Poddar et al., 2005) and BubR1 (Shannon et al., 2002; Rancati et al., 2005) are essential for Cdc20 binding (reviewed in Zhou et al., 2003; Chan and Yen, 2003; Hoyt, 2001). The MCC is considered to be essential for MSAC function: it binds and inhibits the APC/C (Sudakin et al., 2001; Fang et al., 1998; Shannon et al., 2002; Millband and Hardwick, 2002; Acquaviva et al., 2004; Morrow et al., 2005; D’Angiolella et al., 2003; Tang et al., 2001). During metaphase to anaphase transition, the APC/C\textsubscript{Cdc20} catalyzes the ubiquitination of cyclin B and Securin (Peters, 2002), which binds and inhibits the protease Separase (May and Hardwick, 2006). After release of inhibition, separase cleaves the Cohesin subunit Scc1 which breaks the Cohesin ring (Yu and Tang, 2005).
Recently, intensive studies led to an improved understanding of Mad2–Cdc20 binding resulting in two alternative mechanistic models: the “Exchange” (Luo et al., 2004) and the “Template” (DeAntoni et al., 2005) model (for reviews see Lénárt and Peters, 2006; Nasmyth, 2005; Hardwick, 2005; Hagan and Sorger, 2005). Mathematical modeling (Ibrahim et al., 2008b) has shown that the exchange model cannot describe metaphase to anaphase switching properly in contrast to the template model, by which, on the other hand, only about half of the amount of the free Cdc20 in the nucleus is sequestered by Mad2. It thus remains an open question, whether the rest of Cdc20 is complexed by other compounds or the APC/C is blocked by other means.

To build a quantitative model of the MCC, we analysed the MCC and Cdc20 dynamics in more detail. In particular, the role of kinetochore control, e.g. by localization of proteins to the DNA, and possible inhibition and amplification effects have to be studied, as recent studies (Doncic et al., 2005, 2006) have shown that signal propagation and stability in three dimensions depends heavily on the latter. Here, we investigated MCC formation, mainly based on Mad2:Cdc20 complexation, which can be regarded as a seeding reaction. Using our results (Ibrahim et al., 2008b) on the template model, we derived mathematical models for MCC complex assembly, applying results from cell-biological experiments. We analysed the quantitative properties of two different variants, the “kinetochore-dependent model” (KDM) and the “kinetochore-independent model” (KIM) with different biochemical control mechanisms of reactions related to kinetochore associated proteins. Furthermore, we extended the KDM by signal amplification by an additional two step catalysis process (DeAntoni et al., 2005), and by inhibition by p31*omni* (Xia et al., 2004). In particular, we show that, based on experimentally determined data, effective MCC formation is not combined with complete Cdc20 sequestering.

The MCC might either be an APC/C inhibitor, might act by APC/C sequestration, or by inhibiting Cdc20. New models of APC/C geometry, derived from Cryo EM data and mutant analysis (Passmore et al., 2005; Ohi et al., 2007; Dube et al., 2005; Thornton et al., 2006), suggest molecular mechanisms (Thorton and Toczyski, 2006; Peters, 2006; Yu, 2007). Our modelling results contribute to this discussion. Here, we concentrate on the Cdc20-related aspects and their influence on MCC formation.

2. Molecular biological basis of MCC models

Sudakin et al. (2001) analysed and described the MCC in HeLa cells. It contains Mad2, Bub3, BubR1 and Cdc20 in apparently equal stoichiometries. A similar complex was identified in budding (Hardwick et al., 2000) and fission (Milliband and Hardwick, 2002) yeasts and in Xenopus (Chung and Chen, 2003). Bub3 associates with BubR1 (Sudakin et al., 2001; Taylor et al., 2004, 1998). This interaction is constitutive and is required for the localization of BubR1 to the kinetochores during mitosis. In prometaphase, CENP-E activates the kinase activity of BubR1 at unattached kinetochores (Mao et al., 2003, 2005; Chan et al., 1998). It is unclear whether the BubR1 activation is required for MCC function (Mao et al., 2003; Chen, 2002): the kinase activity of BubR1 might not be required in the MCC, however, it might control other aspects of kinetochore signaling or chromosome alignment (Ditchfield et al., 2003; Lampson and Kapoor, 2005) [reviewed in Musacchio and Salmon, 2007]. BubR1 activity is switched off upon microtubule attachment (Mao et al., 2005; Braunstein et al., 2007).

The binding properties of BubR1 are controversial. BubR1 cannot bind Mad2 directly (Fang, 2002). Though it was reported that BubR1 does not form a ternary complex with Mad2 and Cdc20 (Bolanos-Garcia et al., 2005; Davenport et al., 2006), for budding and fission (Burton and Solomon, 2007; King et al., 2007) yeasts such complexes (with Mad3) were found when investigating the highly conserved KEN boxes. Two Cdc20 binding sites were identified on BubR1 (Bolans-Garcia et al., 2005; Davenport et al., 2006): Binding of the N-terminal region of BubR1 to Cdc20 requires prior binding of Mad2 to Cdc20 (Davenport et al., 2006). The other site (between residues 490 and 560) can bind Cdc20 tightly regardless of Mad2 being bound to Cdc20 (Davenport et al., 2006). Thus, BubR1 can form a ternary complex with Bub3 and Cdc20 which however has no inhibitory activity at the APC/C (unpublished data Sudakin et al., 2001).

During prometaphase, Cdc20 and all SAC proteins concentrate at unattached kinetochores (Cleveland et al., 2003; Maiato et al., 2004), like Mad1 (Campbell et al., 2001; Chung and Chen, 2002), Mad2 (Fang et al., 1998; Lampson and Kapoor, 2005), BubR1 (Morrow et al., 2005; Hoffman et al., 2001), Bub1 (Taylor et al., 1998; Chen, 2002), Bub3 (Taylor et al., 1998; Howell et al., 2004), and Mps1 (Stucke et al., 2004, 2002). Kinetochore localization of Cdc20 and of its binding partners in the MCC is dynamic. Localization of all SAC proteins at unattached kinetochores in mitosis provides a catalytic platform and contributes to MCC formation (Kallio et al., 2002; Howell et al., 2000; Shah et al., 2004). The MCC is also detectable in normal metaphase–arrested cells in which the SAC is inactive. This indicates that MCC formation does not require checkpoint activation (Poddar et al., 2005). Moreover, the MCC is also detectable in checkpoint defective cells (Poddar et al., 2005; Fraschini et al., 2001). A detailed study (Meraldi et al., 2004) proposed that cytosolic Mad2–BubR1 is essential to restrain anaphase onset early in mitosis when kinetochores are still assembling. These arguments support the idea that the MCC (and its subcomplexes) might form in a kinetochore-independent manner (for review see Musacchio and Salmon, 2007). We thus distinguish two dynamical models (see Fig. 1): a KDM, and a KIM. In the following, we define the chemical reaction equations based on empirical results and analyse their properties.

3. Mathematical modeling of the MCC

We analyse different models for MCC function considering in particular the role of the attachment status of the kinetochore. For each model, we describe the reaction equations in the usual biochemical notation specifying kinetic constants and assuming mass action rules to derive the differential equations for the concentrations as functions of time. Some of the reactions are independent of the kinetochore attachment status, others are mediated in some way by attachment or non-attachment, respectively. The most prominent equation will be the formation of the MCC complex

\[
\text{Cdc20:C-Mad2 + Bub3:BubR1} \rightarrow \text{MCC}
\]

which, in the KDM, proceeds only, when the kinetochore is unattached, whereas in the KIM it proceeds all the time independently of the attachment status. Therefore, in the KDM, we set \(k_F := k_u \cdot u\), where \(u\) is a switching parameter, which is set to \(u = 1\) as long as the kinetochore is unattached and switches to \(u = 0\) when it attaches. The backward reaction with kinetic parameter \(k_{-F} := k_{-u}\) proceeds all the time. In the KIM, on the contrary, we assume no dependency of the forward reaction on the attachment status and set \(k_F := k_u\). In general, more than one equation will be affected by the kinetochore attachment status, and they will be all regulated by \(u\). We do not consider down or upregulation by a certain percentage, in agreement with experimental findings (Vink et al., 2006; Musacchio and Salmon, 2007).
Fig. 1. Schematic networks of MCC models. Black lines represent the kinetochore-dependent (KDM) and the kinetochore-independent (KIM) models. The KDM is obtained for \( u \) inside the green circles being \( u = 1 \) for phase 1 before kinetochore attachment, and \( u = 0 \) for phase 2 after attachment. In the KIM, in contrast, \( u \) inside the green circles is \( u = 1 \) in both phases. The red lines represent the amplification reactions as defined in Section 4.3. For biochemical details see text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)


ing with attached kinetochores only, we use the factor \( v = 1 - u \), as is the case in the model with \( p_{31}\text{comet}_\text{contribution} \).

Biologically, the switching parameter \( u \) represents the function of dynein, which after microtubule attachment removes the Mad1:C–Mad2 2:2 complex from the kinetochore site. It might also represent potential additional functions which contribute to switching behavior.

From the reaction equations, ordinary differential equations (ODEs) are derived using mass action kinetics. For a set of given initial concentrations for all reaction partners, the ODEs are integrated until steady state is reached before attachment (phase 1). Then \( u \) switches from 1 to 0 and the resulting set of equations is again integrated until steady state is reached (phase 2). For consistency and comparability of all models we use the same initial concentrations (c.f. Table 1). The actual values are chosen according to data from the literature. When only a certain range was known, we have used different starting values for our computations, but we did not find qualitatively differing trajectories. The kinetic constants are also taken from literature as far as they are known (Table 2). For all other constants (\( k_2, k_3, k_5, k_7, k_8 \), see Table 2), several computations with values representing the whole physiologically reasonable parameter range are compared. The trajectories are discussed in corresponding figures. They all show a continuous dependence of the parameters used. Criteria for

<table>
<thead>
<tr>
<th>Species</th>
<th>Initial concentration (M)</th>
<th>Comments and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdc20</td>
<td>( 2.2 \times 10^{-7} )</td>
<td>Fang (2002), Howell et al. (2000) and Tang et al. (2001)</td>
</tr>
<tr>
<td>O-Mad2</td>
<td>( 1.3 \times 10^{-7} )</td>
<td>Fang (2002), Howell et al. (2000) and Tang et al. (2001)</td>
</tr>
<tr>
<td>Mad1:C-Mad2</td>
<td>( 0.5 \times 10^{-7} )</td>
<td>Luo et al. (2004), Fang (2002) and Howell et al. (2000)</td>
</tr>
<tr>
<td>Bub3:BubR1</td>
<td>( 1.3 \times 10^{-7} )</td>
<td>Fang (2002) and Tang et al. (2001)</td>
</tr>
<tr>
<td>( p_{31}\text{comet} )</td>
<td>( 1 \times 10^{-6} )</td>
<td>Xia et al. (2004) and Mapelli et al. (2006)</td>
</tr>
</tbody>
</table>

Initial concentrations of other species are zero.

Table 1

Initial concentrations for integration

Table 2

Kinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value or range</th>
<th>Comments and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 )</td>
<td>( 2 \times 10^3 \text{M}^{-1} \text{s}^{-1} )</td>
<td>Vink et al. (2006)</td>
</tr>
<tr>
<td>( k_{-1} )</td>
<td>( 0.2 \text{s}^{-1} )</td>
<td>Vink et al. (2006)</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>( 10^3, 10^6, 10^9, 10^{10} \text{M}^{-1} \text{s}^{-1} )</td>
<td>Ibrahim et al. (2008b)</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>( 10^{-3}, 10^{-2}, 10^{-1} \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_4 )</td>
<td>( 10^4 \text{M}^{-1} \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_{-4} )</td>
<td>( 0.02 \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_5 )</td>
<td>( 10^4, 10^5 \text{M}^{-1} \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_{-5} )</td>
<td>( 0.2 \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_6 )</td>
<td>( 10^3 \text{M}^{-1} \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_7 )</td>
<td>( 10^3 \text{M}^{-1} \text{s}^{-1} )</td>
<td>Musacchio and Salmon (2007)</td>
</tr>
<tr>
<td>( k_{-7} )</td>
<td>( 0.2 \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_8 )</td>
<td>( 10^3 \text{M}^{-1} \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_{-8} )</td>
<td>( 0.3 \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_7^p )</td>
<td>( 0.037 \text{s}^{-1} )</td>
<td>Vink et al. (2006)</td>
</tr>
<tr>
<td>( k_8^p )</td>
<td>( 10^3 \text{M}^{-1} \text{s}^{-1} )</td>
<td>This study</td>
</tr>
<tr>
<td>( k_{-8}^p )</td>
<td>( 0.3 \text{s}^{-1} )</td>
<td>This study</td>
</tr>
</tbody>
</table>
comparison of the ability of the model to show acceptable switching behavior are the level of MCC depletion and Cdc20 increase and the recovery time after the metaphase to anaphase transition.

To solve the non-linear systems of highly stiff ODEs, which arise besides non-stiff systems, a variety of computational approaches have been applied. In particular, we use high order (seven–eighth) Runge–Kutta (Fehlberg and Prince) formulas, a modified Rosenbrock formula, variable order Adams–Bashforth–Moulton, backward differentiation formulas, and an implicit Runge–Kutta formula. We switch between these methods automatically according to the system’s stiffness state. We implemented our code in MATLAB (2007). The numerical results were also validated with Copasi (Hoops et al., 2006).

4. Dynamics of different MCC models

4.1. Kinetochore-dependent MCC model (KDM)

The template model (DeAntoni et al., 2005) for the Cdc20:C-Mad2 complex formation can be regarded as a seeding reaction to MCC assembly. In our recent mathematical description and sim-

Fig. 2. Kinetochore-dependent MCC model (KDM). For three different $k_3$-values, the time evolution within phase 1 before attachment (0–2000 s) and within phase 2 after attachment (2000–4000 s) of Cdc20 (a1–c1) and MCC (a2–c2) are shown for five different $k_2$-values (colored lines). Solutions are nearly independent of $k_2$ for $k_2 \geq 10^7 \text{M}^{-1} \text{s}^{-1}$. Whereas switching behavior for MCC is satisfactory for any $k_3$, Cdc20 recovery time becomes short enough only for $k_3 \geq 0.01 \text{s}^{-1}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)
Fig. 3. Species concentrations for KDM (a) and KIM (b). For $k_2 = 10^7 \text{ M}^{-1}\text{s}^{-1}$ and $k_3 = 0.01 \text{s}^{-1}$, the time evolution of all eight species in the MCC assembly models are shown before (phase 1, $t = 0–2000$ s) and after attachment (phase 2, $t = 2000–4000$ s). Bub3:BubR1 is not totally used up to sequester Cdc20. Mad1:C-Mad2 shows its function as a catalyzer for Cdc20:C-Mad2 production. Time evolution is identical in KDM (a) and KIM (b) before $t = 2000$ s, but quantitatively and qualitatively (Cdc20:C-Mad2) different after that. Details see text. (a) Kinetochore-dependent model (KDM). (b) Kinetochore-independent model (KIM).

ulation analysis of the template model (Ibrahim et al., 2008b), we found that for realistic Mad2 concentrations, Cdc20 cannot be sequestered completely in the cell by Mad2 alone. In addition to binding to Mad2, Cdc20 also binds to BubR1 (Bolanos-Garcia et al., 2005; Davenport et al., 2006; Chen, 2002; Fang, 2002) and it might be this protein which sequesters the remaining part of Cdc20. We therefore added to the reaction equations of the template model (Eqs. (1)–(3)) (Ibrahim et al., 2008b) those reaction equations describing the interaction with BubR1. The reaction scheme is presented in Fig. 1 and the reaction rules (1)–(6) are listed below. The BubR1 reaction Eqs. (4) for MCC formation and (5) for complexation with Bub3 and Cdc20 were deduced from experimental work (Sudakin et al., 2001; Davenport et al., 2006; Fang, 2002). Reaction (6) and its low rate were mentioned by Musacchio and Salmon 2007) (see also Fang, 2002; Davenport et al., 2006).

\begin{align}
\text{Mad1:C-Mad2} + \text{O-Mad2} & \equiv \text{Mad1:C-Mad2:O-Mad}^* \\
& \rightarrow \text{Mad1:C-Mad2:O-Mad}^* (1) \\
\text{Mad1:C-Mad2:O-Mad}^* + \text{Cdc20} & \rightarrow \text{Cdc20:C-Mad2} + \text{Mad1:C-Mad2} (2) \\
\text{Cdc20:C-Mad2} & \rightarrow \text{O-Mad2} + \text{Cdc20} (3) \\
\text{Cdc20}:\text{C-Mad2} + \text{Bub3:BubR1} & \rightarrow \text{Bub3:BubR1:O-Mad}^* (4) \\
\text{Cdc20} + \text{Bub3:BubR1} & \rightarrow \text{Bub3:BubR1:Cdc20} (5)
\end{align}
Fig. 4. Kinetochore-independent MCC model (KIM). Same representation as in Fig. 2. Concentration curves in phase 1 before attachment (0–2000 s) are identical to those in Fig. 2, whereas in phase 2 after attachment (2000–4000 s) the parameter \( u \) (in the green circle in Fig. 1) is still set to \( u = 1 \) (no kinetochore control). As a consequence, switching is very slow. Furthermore, Cdc20 recovery and MCC depletion levels are poor. (a1) \( k_3 = 0.001 \text{ s}^{-1} \). (a2) \( k_3 = 0.001 \text{ s}^{-1} \). (b1) \( k_3 = 0.01 \text{ s}^{-1} \). (b2) \( k_3 = 0.01 \text{ s}^{-1} \). (c1) \( k_3 = 0.1 \text{ s}^{-1} \). (c2) \( k_3 = 0.1 \text{ s}^{-1} \).

The corresponding differential equations are listed in Appendix A, Eqs. (A.1)–(A.8). The first two reactions (1) and (2) are kinetochore controlled and therefore the rates are multiplied by the switching parameter \( u \). In the reaction scheme we assume that also the BubR1 forward reactions (4) and (5) are kinetochore dependent so that also rates \( k_4 \) and \( k_5 \) are multiplied by \( u \). Most concentrations and rates of this reaction scheme were taken from literature (listed in Tables 1 and 2). For \( k_4 \) we chose a high rate of \( 10^7 \text{ M}^{-1} \text{ s}^{-1} \) similar to \( k_1 \) and \( k_2 \) since only for high values of \( k_4 \) high amounts of MCC would be formed. Nevertheless, we analysed the consequences of lower values of \( k_4 \) (see below), \( k_5 \) should be considerably smaller than \( k_4 \) and was chosen to be \( 10^4 \text{ M}^{-1} \text{ s}^{-1} \). Below, also the influence of larger values of \( k_5 \) was studied. The backward reactions \( k_{-4} \) and \( k_{-5} \) should be slow and in the range of the experimental values of \( k_{-1} \); we put \( k_{-4} = 0.02 \text{ s}^{-1} \) and \( k_{-5} = 0.2 \text{ s}^{-1} \) (see Table 2). For these kinetic data, we calculated the time-dependent values of all species concentrations. Here we focus our discussion on the dynamics of the Cdc20 and MCC concentrations. In Fig. 2, the Cdc20
Fig. 5. Amplification effects. Here, $k_3 = 0.01$. For slow (a1, a2) and fast (b1, b2, c1, c2) amplification reaction rates, the concentration curves for Cdc20 and MCC show fast switching behavior in the uncontrolled (a1/2–b1/2) as well as in the controlled (c1–c2) case, where rates $k_7$ and $k_8$ are multiplied by $u$. Only for fast ($k_7 = k_8 = 10^4 \text{M}^{-1} \text{s}^{-1}$) amplification reaction rates in the uncontrolled case, Cdc20 does not recover to the maximal level (b1). (a1) Slow ($k_7 = k_8 = 10^4 \text{M}^{-1} \text{s}^{-1}$). (a2) Slow ($k_7 = k_8 = 10^4 \text{M}^{-1} \text{s}^{-1}$). (b1) Fast ($k_7 = k_8 = 10^6 \text{M}^{-1} \text{s}^{-1}$). (b2) Fast ($k_7 = k_8 = 10^6 \text{M}^{-1} \text{s}^{-1}$). (c1) Fast ($k_7 = k_8 = 10^6 \text{M}^{-1} \text{s}^{-1}$). (c2) Fast ($k_7 = k_8 = 10^6 \text{M}^{-1} \text{s}^{-1}$).

concentration is plotted for three different values of $k_3$ and five different values of $k_2$. With increasing values of $k_3$, we observed faster switching behavior. For $k_3 = 0.01 \text{ s}^{-1}$, switching is already considerably fast, consistent with the findings for the template model. $k_2$ hardly influenced the Cdc20 concentration when being larger than $10^7 \text{M}^{-1} \text{s}^{-1}$. For no parameter value combination of $k_2$ and $k_3$ could Cdc20 be sequestered completely. Instead, for low values of $k_2$ and high values of $k_3$, Cdc20 concentration was reduced to slightly more than half and other parameter value combinations reduced it even less. The MCC shows fast switching behavior in all cases considered (see Fig. 2).

In Fig. 3 a, we show the time course of the concentrations of all eight species in the model for the physiologically relevant parameters $k_2 = 10^7 \text{M}^{-1} \text{s}^{-1}$ and $k_3 = 0.01 \text{ s}^{-1}$. It is interesting to note that [Bub3:BubR1] does not decay to zero to sequester more Cdc20 into MCC. There is also virtually no Bub3:BubR1:Cdc20 formed. Instead, about 15% of the Bub3:BubR1 concentration remains freely floating in the cell plasma. Another interesting feature is exhibited by the Mad1, Mad2, and Cdc20 dynamics. As there is virtually no Mad1:C-Mad2:O-Mad2* formed, this complex shows its property as short living intermediate in the reactions (1) and (2), clearly catalysed by Mad1:C-Mad2, staying at constant concentration of $0.5 \times 10^{-7} \text{M}$.
4.2. Kinetochore-independent MCC model (KIM)

The line of biochemical reactions placing MCC formation under the control of the kinetochore are not clear (reviewed by Musacchio and Salmon, 2007). MCC formation might be kinetochore independent (Poddar et al., 2005; Fraschini et al., 2001; Meraldi et al., 2004). We therefore analysed a modified kinetochore-independent model in which the reaction Eqs. (4) and (5) are now replaced by $u$-independent Eqs. (7) and (8) (see Fig. 1):

\[
\begin{align*}
\text{Cdc20:C-Mad2} + \text{Bub3:BubR1} & \rightleftharpoons \text{MCC} \\
\text{Cdc20+Bub3:BubR1} & \rightleftharpoons \text{Bub3:BubR1:Cdc20}
\end{align*}
\]

The resulting differential equations are the same as in Appendix A, Eqs. (A.1)–(A.8), except for the kinetic constants $k_4 \cdot u$ and $k_5 \cdot u$, which have to be replaced by $k_4$ and $k_5$, respectively. Using the same initial concentrations (Table 1) and rate values (Table 2) as above we calculated the time-dependent model behavior. Of course, until kinetochore attachment, both, time dependence and concentrations are unchanged since $u=1$. After attachment, however, we found very slow switching and Cdc20 and MCC concentration changes only for larger $k_3$. Even for large $k_3$, Cdc20 recovery and MCC depletion were not complete (see Fig. 4). The quantitative failure of this reaction model indicates that the BubR1 reactions are kinetochore controlled.

As already done for the KDM, we show the concentration over time curves for all eight species in the KIM for $k_2 = 10^7$ M$^{-1}$ s$^{-1}$ and $k_3 = 0.01$ s$^{-1}$ in Fig. 3b. The characteristic features of the catalyzing reactions (1) and (2) involving Mad1 remain the same, but there is a drastic qualitative change in the concentration dynamics of Cdc20:C-Mad2, which decreases immediately after switching, because without kinetochore control it still restores MCC (Eq. (7)) besides decaying into its components (Eq. (3)). There is thus not only a quantitative change resulting in a slower dynamics, but also a qualitative change in the time evolution of the trajectory of [Cdc20:C-Mad2].

4.3. Amplification effects

DeAntoni et al. (2005) hypothesized that in analogy to the reactions based on Mad1 (Eqs. (1) and (2)), Cdc20:C-Mad2 can also be formed by reactions catalyzed by O-Mad2 and Cdc20 in a two step mechanism as described by the two reaction Eqs. (9) and (10). This additional pathway for the production of Cdc20:C-Mad2 results in a signal amplification for the SAsC since Cdc20:C-Mad2 now is produced not only at the location of Mad1 at the kinetochore but at a larger number of locations everywhere in the cell. We checked
Fig. 7. Maximal Cdc20 sequestering through increase of free Mad2. The dynamical behavior of the KDM showing the time evolution of [Cdc20] (a1–c1) and [MCC] (a2–c2) for the same values of $k_2$ and $k_3$ as in Fig. 2. In contrast, here the Mad2 initial level is increased eightfold. Cdc20 concentration decreases to zero for low $k_3$ and high $k_2$, while the MCC concentration increases slightly independent of $k_1$. (a1) $k_3 = 0.001 \text{s}^{-1}$. (a2) $k_3 = 0.001 \text{s}^{-1}$, (b1) $k_3 = 0.01 \text{s}^{-1}$, (b2) $k_3 = 0.01 \text{s}^{-1}$, (c1) $k_3 = 0.1 \text{s}^{-1}$, (c2) $k_3 = 0.1 \text{s}^{-1}$.

When calculating the time-dependent Cdc20 and MCC concentrations, we observed hardly any change for small values of $k_7$ and $k_8$ (see Fig. 5, a1–a2) compared to the KDM (Fig. 2, b1–b2). This result was expected since for small rates, reactions (9) and (10) do not contribute to the model behavior. When, however, the rates adopt values in the realistic range ($k_7 = k_8 = 10^6 \text{ M}^{-1} \text{s}^{-1}$, see Table 2), we observed only little Cdc20 recovery after kinetochore attachment (see Fig. 5, b1–b2). This result is due to both reactions being independent of kinetochore control. The MCC concentration is hardly influenced by these reactions, since they do not contribute to MCC regulation.

Cdc20·C-Mad2 + O-Mad2 $\xrightleftharpoons[k_7]{k_3}^{\rightarrow} \text{Cdc20·C-Mad2·O-Mad2}$  

When calculating the time-dependent Cdc20 and MCC concentrations, we observed hardly any change for small values of $k_7$ and $k_8$ (see Fig. 5, a1–a2) compared to the KDM (Fig. 2, b1–b2). This result was expected since for small rates, reactions (9) and (10) do not contribute to the model behavior. When, however, the rates adopt values in the realistic range ($k_7 = k_8 = 10^6 \text{ M}^{-1} \text{s}^{-1}$, see Table 2), we observed only little Cdc20 recovery after kinetochore attachment (see Fig. 5, b1–b2). This result is due to both reactions being independent of kinetochore control. The MCC concentration is hardly influenced by these reactions, since they do not contribute to MCC regulation.

Cdc20·C-Mad2 + O-Mad2 $\xrightleftharpoons[k_7]{k_3}^{\rightarrow} \text{Cdc20·C-Mad2·O-Mad2}$  

Cdc20·C-Mad2 + O-Mad2 $\xrightleftharpoons[k_7]{k_3}^{\rightarrow} \text{Cdc20·C-Mad2}$  

the effect of this assumption by adding two additional reactions to the template model, but we found no improvement of the template model behavior (Ibrahim et al., 2008b). Also here, we added the two reaction Eqs. (9) and (10) to the KDM and analysed the influence of the amplification on the model (see Fig. 1, red arrows).

Cdc20·C-Mad2 + O-Mad2 $\xrightleftharpoons[k_7]{k_3}^{\rightarrow} \text{Cdc20·C-Mad2·O-Mad2}$  

Cdc20·C-Mad2 + O-Mad2 $\xrightleftharpoons[k_7]{k_3}^{\rightarrow} \text{Cdc20·C-Mad2}$  

Cdc20·C-Mad2 + O-Mad2 $\xrightleftharpoons[k_7]{k_3}^{\rightarrow} \text{Cdc20·C-Mad2}$
It might be speculated that also the amplification reactions might be somehow kinetochore dependent. When multiplying the rates \( k_7 \) and \( k_9 \) by a switching parameter \( u \) (controlled case), we observed the same model behavior (see Fig. 5, c1–c2 for fast amplification reaction) as for the KDM without amplification (Fig. 2, b1–b2). For slow amplification reactions, the concentration curves (figures not shown) are virtually identical to those in the uncontrolled case (Fig. 5, a1–a2).

4.4. \( p31^{\text{comet}} \) contributions

The negative spindle checkpoint regulator \( p31^{\text{comet}} \) is a Mad2 ligand. Its negative effect on the MSAC is based on its competition with O-Mad2 for C-Mad2 binding (Habu et al., 2002; Xia et al., 2004; Mapelli et al., 2006; Yu, 2006). It forms triple complexes with C-Mad2 and either Mad1 or Cdc20 (Xia et al., 2004; Vink et al., 2006; Mapelli et al., 2006). In our analysis of the template model (Ibrahim et al., 2008b), we added a reaction describing the effect of \( p31^{\text{comet}} \). We could show that, when \( p31^{\text{comet}} \) is activated at microtubule attachment to the kinetochores, it can function as a cellular factor contributing to the checkpoint switching behavior: \( p31^{\text{comet}} \) function can replace the switching parameter \( u \). Here, we introduced the same \( p31^{\text{comet}} \) reactions (17) and (18) to the KDM:

\[
\text{Mad1:C-Mad2} + O-Mad2 \xrightarrow{k_1} \text{Mad1:C-Mad2:O-Mad2}^* \quad (11)
\]

\[
\text{Mad1:C-Mad2} + \text{Cdc20} \xrightarrow{k_2} \text{Cdc20:C-Mad2} + \text{Mad1:C-Mad2} \quad (12)
\]

\[
\text{Cdc20:C-Mad2} \xrightarrow{k_3} \text{O-Mad2} + \text{Cdc20} \quad (13)
\]

\[
\text{Cdc20}:\text{C-Mad2} + \text{Bub3}:\text{BubR1} \xrightarrow{k_4 u} \text{MCC} \quad (14)
\]

\[
\text{Cdc20} + \text{Bub3}:\text{BubR1} \xrightarrow{k_5 u} \text{Bub3}:\text{BubR1}:\text{Cdc20} \quad (15)
\]

\[
\text{Cdc20} + \text{O-Mad2} \xrightarrow{k_6} \text{Cdc20:C-Mad2} \quad (16)
\]

\[
\text{Mad1}:\text{C-Mad2} + p31v^* \xrightarrow{k_7 p} \text{Mad1}:\text{C-Mad2}:p31 \quad (17)
\]

\[
\text{Cdc20}:\text{C-Mad2} + p31v^* \xrightarrow{k_8 p} \text{Cdc20}:\text{C-Mad2}:p31 \quad (18)
\]

This corresponds to the differential equations (C.1)–(C.11) in Appendix C, where \( v = 1 - u \). Reaction (11) and (12) are now kinetochore uncontrolled (no multiplication of the rates by \( u \)). However, MCC formation (14) and the BubR1 reaction (15) are not linked to \( p31^{\text{comet}} \) function, so that these reactions remain kinetochore controlled with rates multiplied by the switching parameter \( u \). Thus, the reaction scheme (Eqs. (11)–(18)) remains kinetochore dependent. We studied the influence of \( p31^{\text{comet}} \) on the behavior of this model and observed, as for the template model (Ibrahim et al., 2008b), that \( u \) in reactions (1) and (2) can be replaced by \( p31^{\text{comet}} \) function without changing the behavior of Cdc20 and MCC (data not shown). In this respect, the switching parameter \( u \) additionally represents \( p31^{\text{comet}} \) function and further potentially unidentified reactions by additional proteins. An example could be UbcH10 (Townsley et al., 1997), which plays a role in checkpoint inactivation (Reddy et al., 2007). Also other effects like protein activation, inhibition, or degradation might contribute to \( u \).

When keeping the original KDM (with reactions (1) and (2) controlled by \( u \), instead of uncontrolled (11) and (12)) and including the \( p31^{\text{comet}} \) contribution (Eqs. (17) and (18)), the system behavior virtually does not change (data not shown).

4.5. Maximum sequestering of Cdc20

For experimentally determined parameter values, Cdc20 cannot be totally sequestered in the MCC models analysed. Cdc20 can be strongly reduced for high values of \( k_5 \). A simulation for \( k_5 = 10^8 \text{ M}^{-1} \text{s}^{-1} \) showed that most of BubR1:Bub3 binds Cdc20 and removes it from the cell plasma. Fig. 6 displays the low Cdc20 concentrations for low values of \( k_3 \) and high values of \( k_2 \) as well as the fast switching behavior of the model. However, most of BubR1:Bub3 is used-up for this reaction so that hardly any MCC can form (see Fig. 6, a2, for the lowest value of \( k_3 \)). For higher \( k_3 \), there is virtually no MCC assembled. Thus, for high values of \( k_5 \), strong Cdc20 sequestering is combined with MCC depletion which would leave the APC/C uncontrolled. On the other hand, maximum MCC concentration was obtained for low values of \( k_5 \); in this case, most BubR1:Bub3 contributes to MCC formation so that hardly any BubR1:Bub3:Cdc20 can form (data not shown).

In order to investigate another possibility to increase Cdc20 sequestration, we changed initial conditions. As shown in in vitro assays (Fang, 2002), complete Cdc20 binding is obtained when the O-Mad2 initial concentration is increased to at least eightfold beyond experimentally measured physiological values. Fig. 7 shows that indeed for eightfold higher O-Mad2 concentrations, the Cdc20 concentration decreased down to zero for low values of \( k_3 \) and high

![Fig. 8. KDM with eightfold increased BubR1:Bub3 initial concentration for \( k_5 = 0.1 \text{ s}^{-1} \). Same representation as in Fig. 2. The comparison shows that BubR1:Bub3 initial concentration has no major influence on Cdc20 sequestration.](image-url)
Fig. 9. KDM with twofold increased initial concentration of Mad2 (a), Mad2 and BubR1:Bub3 (b), and BubR1:Bub3 (c), for $k_3 = 0.01 \text{ s}^{-1}$. Same representation as in Fig. 2. The comparison shows that an increase of BubR1:Bub3 or Mad2 initial concentration alone has no major influence on Cdc20 sequestration, whereas a twofold increase of both concentrations shows major effects. (a1) Twofold Mad2 concentration. (a2) Twofold Mad2 concentration. (b1) Twofold Mad2 and BubR1:Bub3. (b2) Twofold Mad2 and BubR1:Bub3. (c1) Twofold BubR1:Bub3 concentration. (c2) Twofold BubR1:Bub3 concentration.

Values of $k_2$ in our simulation of the KDM, while the MCC concentration slightly increased independently of $k_3$. The MCC concentration increased since under these conditions more Cdc20:Mad2 is available. The concentration increase is limited due to limitations in the BubR1:Bub3 concentration.

Thus, for the assumed values of $[\text{Bub3:BubR1}] = 1.3 \times 10^{-7} \text{ M}$ and $[\text{Cdc20}] = 2.2 \times 10^{-7} \text{ M}$ (Table 1), MCC cannot sequester Cdc20 totally. In addition, the formation of Bub3:BubR1:Cdc20 (Eq. (5)) and Cdc20:C-Mad2 by the two step catalysis (Eqs. (1) and (2)) and the direct reaction (Eq. (6)) are not well suited to sequester a considerable amount of Cdc20, as shown in Fig. 3 a. We therefore studied the effect of the initial Bub3:BubR1 concentration on the system by increasing it eightfold to $10.4 \times 10^{-7} \text{ M}$. The result for $k_3 = 0.1 \text{ s}^{-1}$ is shown in Fig. 8. Comparing it to Fig. 2 with $[\text{Bub3 : BubR1}] = 1.3 \times 10^{-7} \text{ M}$, we see a slightly stronger Cdc20 sequestering down to about 45% of the initial concentration for $k_2 \geq 10^7$. [MCC] attains its maximal level of about $1.3 \times 10^{-7} \text{ M}$, which does not rise any further, even when the Bub3:BubR1 initial concentration is further increased. For $k_3 = 0.01$ and $0.001 \text{ s}^{-1}$, the sequestration increase is less than 10% and thus negligible (data

Fig. 10. KDM with twofold increased initial concentration of Mad2 (a), Mad2 and BubR1:Bub3 (b), and BubR1:Bub3 (c), for $k_3 = 0.01 \text{ s}^{-1}$. The concentrations for all species are shown for the whole simulation time. (a) Twofold Mad2 concentration. (b) Twofold Mad2 and BubR1:Bub3. (c) Twofold BubR1:Bub3 concentration. Not shown). Likewise, for these parameter values, the MCC maximal concentration and dynamics hardly change. The corresponding values in Fig. 2 are dependent on $k_3$. Also, the switching behavior for both Bub3:BubR1 concentrations is comparable. Altogether, we conclude that the Bub3:BubR1 initial concentration alone has no major influence on Cdc20 sequestration.

As mentioned above, BubR1:Bub3 concentration limits MCC concentration increase caused by higher O-Mad2 initial concentrations. To investigate this relationship in more detail, we increased both O-Mad2 as well as BubR1:Bub3 initial concentrations twofold. The result for $k_3 = 0.01 \text{ s}^{-1}$ is displayed in Fig. 9 for Cdc20 and MCC dynamics and in Fig. 10 for the concentrations of all components. For twofold increased O-Mad2 concentration alone, Cdc20 sequestration is increased (cf. Fig. 2) but does not reach the same level as for eightfold increased [O-Mad2] (cf. Fig. 7). The MCC maximal concentration, on the contrary, is the same as for eightfold increased [O-Mad2], with slightly slower dynamics. The maximal concentration levels and dynamics for twofold increased [BubR1:Bub3] alone (Fig. 9c) do not differ from the effects for eightfold increased [BubR1:Bub3] (Fig. 8). For the twofold increase of both, [O-Mad2] and [BubR1:Bub3], and for high $k_2$, all Cdc20 can be sequestered and [MCC] gets maximal (Fig. 9b). Almost all Cdc20 is bound in the MCC (Fig. 10). In this respect, the simultaneous doubling of the initial concentrations of O-Mad2 and BubR1:Bub3 shows a non-linear effect.

5. Discussion and conclusions

Building on our investigation (Ibrahim et al., 2008b) of different models for Cdc20:Mad2 complex formation, we have extended the mathematical description of the template model by those reaction equations, which describe Cdc20 sequestration. The major role is played by Bub3:BubR1 and the MCC, which, in turn, blocks the APC/C. We analysed MCC formation, distinguishing a KDM and a KIM. The latter failed to describe correct metaphase to anaphase switching. Presently there are no direct experimental hints for a kinetochore dependence of the Bub3:BubR1 related processes (parameters $k_4$ and $k_5$). Our calculations indicate, however, that a kinetochore dependence of some kind is required. Additional experimental investigations should clarify this situation.

Considering the KDM under realistic conditions, Cdc20 is not sequestered completely but instead remains in the cell plasma to a considerable amount. Complete Cdc20 binding is either attained for unrealistically high concentrations of Mad2 or for high rate values of BubR1:Bub3:Cdc20 formation; the latter, however, sacrificing MCC formation and thus APC/C control. The behavior of the KDM is virtually independent of the Bub3:BubR1 initial concentration. Amplification effects or a detailed description of p31comet contribution did not improve the situation. These results indicate that, on the basis of the reactions considered here, using experimentally determined kinetic constants and initial concentrations, the MSAC regulation does not function by complete Cdc20 sequestration.

An interesting non-linear effect, however, was found when deviating form literature values for more than one constant: the combination of doubling the initial concentrations of Mad2 and BubR1:Bub3 simultaneously showed a maximal Cdc20 sequestration for intermediate formation rates and high decay rates of the Cdc20:C-Mad2 complex. Protein concentration estimations in the cell might well be precise only within a factor of two; we consider it rather improbable, however, that the concentrations of two related species are experimentally determined wrongly in parallel. Nevertheless, assuming twofold higher concentrations for Mad2 and BubR1:Bub3 in contrast to published measured values would indeed allow for an APC/C control mechanism based on complete Cdc20 sequestration. This control mechanism, however, would be unstable since our calculations clearly indicate that Cdc20 sequestration would be dissolved as soon as one of the two concentrations is reduced by a factor of two. We are convinced that essential cellular processes as APC/C control would not be regulated by unstable mechanisms. Thus, either further yet unknown reactions contribute to stabilise the situation, or, confirming our statement from above, the APC/C is not controlled by complete Cdc20 sequestration.

Extending previous work (Sear and Howard, 2006; Doncic et al., 2005) based on partial differential equations, Doncic et al. (2006) showed in an analysis based on ordinary differential equations that
MSAC can act through Cdc20 sequestration. Investigating statistical error propagation in two models describing abstract kinetochore mediated inhibition mechanisms, they exclude that Cdc20 is inhibited solely by protein degradation. However, both mechanisms, sequestration and degradation, can act in parallel showing almost linear behavior in combination. Their model includes only two species, Cdc20 and an abstract signalling complex m which could be Mad2 or another sequestering molecule. This comes very close to our analysis (Ibrahim et al., 2008b) of the template model, where we found complete sequestering of Cdc20 as confirmed by in vitro assays (Fang, 2002), but only for unphysiological parameter sets. Comparing their work and the simulations here, we cannot yet finally conclude on the mechanism of MCC:APC/C function. Nevertheless, diffusion seems to play a subordinate role, as it seems to be very fast as compared to the other reaction rates involved (Doncic et al., 2006; Ibrahim et al., 2007). Instead, the reaction mechanism plays the main role. Whereas Doncic et al. (2006) argue that MSAC can act through Cdc20 sequestration, we described such a process by detailed reactions which show no complete Cdc20 sequestration for measured parameter settings. Based on our current knowledge, thus, complete Cdc20 sequestration is not realised.

Which complexes Bub3:BubR1 is able to form, is a crucial question for MCC formation. It is a general problem of simulation studies to identify all reaction schemes and to incorporate them with the correct reaction kinetics. In general, our study can help to falsify assumptions, but can hardly proof the correctness of a pathway (Popper, 1935). In our analysis, we have found that the KIM is very unlikely to describe MCC assembly, but that the KDM could be a basis for further pathway design.

In our model, we analysed the influence of Cdc20 sequestration on MSAC operation (Musacchio and Salmon, 2007). In how far partial inhibition of the APC/C by the MSAC is tolerable by the cell cycle control, is currently unclear. As the basic reactions, described here and previously (Ibrahim et al., 2008b), for the experimentally determined values, do not lead to complete Cdc20 sequestration, we speculate that the MCC might bind and completely block the APC/C. APC/C activation might then be a consequence of a MCC:APC/C complex modification or rearrangement. To investigate the further reaction scheme for checkpoint function, we have integrated the KDM investigated here into a model describing APC/C inhibition and APC/C/Cdc20 formation after metaphase to anaphase transition (Ibrahim et al., 2008a), which might serve as a basis for more complex investigations. The “tightness” of the APC/C control might be a topic of these future studies.

Acknowledgements

We would like to thank the German Academic Exchange Service (DAAD, grant A04/31166), the Federal Ministry of Education and Research (BMBF, grant 0312704A), and EU (ESIGNET, project no. 12789) for financial support. We also thank Christian Hoischen and the reviewer for their detailed helpful comments and suggestions.

Appendix A. ODEs for “Kinetochore-dependent model” KDM

\[ \frac{d[Mad1:C-Mad2]}{dt} = -k_1 \cdot u[Mad1:C-Mad2][O-Mad2] + k_{-1}[Mad1:C-Mad2:O-Mad2^*] + k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] \] (A.1)

\[ \frac{d[O-Mad2]}{dt} = -k_1 \cdot u[Mad1:C-Mad2][O-Mad2] + k_{-1}[Mad1:C-Mad2:O-Mad2^*] + k_3[Cdc20:C-Mad2] - k_5[Cdc20][O-Mad2] \] (A.2)

\[ \frac{d[Mad1:C-Mad2:O-Mad2^*]}{dt} = k_1 \cdot u[Mad1:C-Mad2][O-Mad2] - k_{-1}[Mad1:C-Mad2:O-Mad2^*] - k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] \] (A.3)

\[ \frac{d[Cdc20]}{dt} = -k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] + k_3[Cdc20:C-Mad2] - k_5 \cdot u[Cdc20][Bub3:BubR1] + k_{-5}[Bub3:BubR1:Cdc20] - k_6[Cdc20][O-Mad2] \] (A.4)

\[ \frac{d[Cdc20:C-Mad2]}{dt} = k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] - k_3[Cdc20:C-Mad2] - k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] + k_6[Cdc20][O-Mad2] \] (A.5)

\[ \frac{d[Bub3:BubR1]}{dt} = -k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] - k_5 \cdot u[Cdc20][Bub3:BubR1] + k_{-5}[Bub3:BubR1:Cdc20] \] (A.6)

\[ \frac{d[MCC]}{dt} = +k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] - k_{-4}[MCC] \] (A.7)

\[ \frac{d[Bub3:BubR1:Cdc20]}{dt} = k_5 \cdot u[Cdc20][Bub3:BubR1] - k_{-5}[Bub3:BubR1:Cdc20] \] (A.8)
Appendix B. ODEs for amplification effects

\[ \frac{d[Mad1:C-Mad2]}{dt} = -k_1 \cdot u[Mad1:C-Mad2][O-Mad2] + k_{-1}[Mad1:C-Mad2:O-Mad2^*] + k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] \] (B.1)

\[ \frac{d[O-Mad2]}{dt} = -k_1 \cdot u[Mad1:C-Mad2][O-Mad2] + k_{-1}[Mad1:C-Mad2:O-Mad2^*] + k_3[Cdc20:C-Mad2] - k_6[Cdc20][O-Mad2] - k_7[Cdc20:C-Mad2][O-Mad2^*] \] (B.2)

\[ \frac{d[Mad1:C-Mad2:O-Mad2^*]}{dt} = k_1 \cdot u[Mad1:C-Mad2][O-Mad2] - k_{-1}[Mad1:C-Mad2:O-Mad2^*] - k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] \] (B.3)


\[ \frac{d[Cdc20:C-Mad2]}{dt} = k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] - k_3[Cdc20:C-Mad2] - k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] + k_6[Cdc20][O-Mad2] - k_7[Cdc20:C-Mad2][O-Mad2^*] + k_{-7}[Cdc20:C-Mad2:O-Mad2^*] \] (C.3)

\[ \frac{d[Cdc20:C-Mad2]}{dt} = k_2 \cdot u[Mad1:C-Mad2:O-Mad2^*][Cdc20] - k_3[Cdc20:C-Mad2] - k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] + k_6[Cdc20][O-Mad2] - k_7[Cdc20:C-Mad2][O-Mad2^*] + k_{-7}[Cdc20:C-Mad2:O-Mad2^*] \] (C.4)

\[ \frac{d[Bub3:BubR1]}{dt} = -k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] - k_5 \cdot u[Cdc20][Bub3:BubR1] - k_5[Bub3:BubR1:Cdc20] \] (C.6)

Appendix C. ODEs for p31comet contribution

\[ \frac{d[Mad1:C-Mad2]}{dt} = -k_1[Mad1:C-Mad2][O-Mad2] + k_{-1}[Mad1:C-Mad2:O-Mad2^*] + k_2[Mad1:C-Mad2:O-Mad2^*][Cdc20] - k_7 \cdot v[Mad1:C-Mad2][p31comet] + k_{-7}[Mad1:C-Mad2:p31comet] \] (C.1)

\[ \frac{d[O-Mad2]}{dt} = -k_1[Mad1:C-Mad2][O-Mad2] + k_{-1}[Mad1:C-Mad2:O-Mad2^*] + k_3[Cdc20:C-Mad2] - k_6[Cdc20][O-Mad2] \] (C.2)

\[ \frac{d[Mad1:C-Mad2:O-Mad2^*]}{dt} = k_1[Mad1:C-Mad2][O-Mad2] - k_{-1}[Mad1:C-Mad2:O-Mad2^*] - k_2[Mad1:C-Mad2:O-Mad2^*][Cdc20] \] (C.3)

\[ \frac{d[Cdc20]}{dt} = -k_2[Mad1:C-Mad2:O-Mad2^*][Cdc20] + k_3[Cdc20:C-Mad2] - k_5 \cdot u[Cdc20][Bub3:BubR1] + k_{-5}[Bub3:BubR1:Cdc20] - k_6[Cdc20][O-Mad2] \] (C.4)

\[ \frac{d[Cdc20:C-Mad2]}{dt} = k_2[Mad1:C-Mad2:O-Mad2^*][Cdc20] - k_3[Cdc20:C-Mad2] - k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] + k_6[Cdc20][O-Mad2] - k_8 \cdot v[Cdc20:C-Mad2][p31comet] + k_{-8}[Cdc20:C-Mad2:p31comet] \] (C.5)

\[ \frac{d[Bub3:BubR1]}{dt} = -k_4 \cdot u[Cdc20:C-Mad2][Bub3:BubR1] + k_{-4}[MCC] - k_5 \cdot u[Cdc20][Bub3:BubR1] - k_5[Bub3:BubR1:Cdc20] \] (C.6)
\[
\frac{d[MCC]}{dr} = +k_4 \cdot [Cdc20:C-Mad2][Bub3:BubR1] - k_4 [MCC]
\] (C.7)

\[
\frac{d[Bub3:BubR1:Cdc20]}{dr} = k_5 \cdot [Cdc20][Bub3:BubR1] - k_5 [Bub3:BubR1:Cdc20]
\] (C.8)

\[
\frac{dp31comtet}{dr} = -k_7 \cdot p\cdot [Mad1:C-Mad2:p31comtet] + k_{-7p}[Mad1:C-Mad2:p31comtet] - k_{-8p}[Cdc20:C-Mad2:p31comtet] + k_{-8p}[Cdc20:C-Mad2:p31comtet]
\] (C.9)

\[
\frac{d[Mad1:C-Mad2:p31comtet]}{dr} = k_{7p} \cdot p\cdot [Mad1:C-Mad2:p31comtet] - k_{-7p}[Mad1:C-Mad2:p31comtet]
\] (C.10)

\[
\frac{d[Cdc20:C-Mad2:p31comtet]}{dr} = k_{8p} \cdot p\cdot [Cdc20:C-Mad2:p31comtet] - k_{-8p}[Cdc20:C-Mad2:p31comtet]
\] (C.11)

References


