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

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Eukaryotic cells rely on a surveillance mechanism, the “Spindle Assembly Checkpoint” ^{M}SAC 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 ^{M}SAC might function by an MCC:APC/C complex rearrangement.

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1. Introduction

Cell division requires well controlled chromosome segregation into the two daughter cells. In mitosis, the “Spindle Assembly Checkpoint” ^{M}SAC (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 ^{M}SAC 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^{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 ^{M}SACfunction: 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^{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).

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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 ^{M}SAC, 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 catalysation process (DeAntoni et al., 2005), and by inhibition by p31^{comet} (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 (Thornton 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

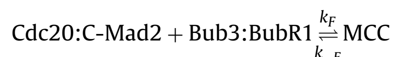
Sudakin et al. (2001) analysed and described the MCC in HeLa cells. It contains Mad2, Bub3, BubR1 and Cdc20 in apparently equal stoichiometries. A similar complex was identified in budding (Hardwick et al., 2000) and fission (Millband and Hardwick, 2002) yeasts and in Xenopus (Chung and Chen, 2003). 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 ^{M}SAC 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 controverse. 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 fission and budding (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 (Bolanos-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 ^{M}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 ^{M}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 ^{M}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) proposes 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



$$\text{Cdc20:C-Mad2} + \text{Bub3:BubR1} \underset{k_{-F}}{\overset{k_F}{\rightleftharpoons}} \text{MCC}$$

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 $k_F{:=}k_4 \cdot u$, 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 $k_{-F}{:=}k_{-4}$ proceeds all the time. In the KIM, on the contrary, we assume no dependency of the forward reaction on the attachment status and set $k_F{:=}k_4$. 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). For regulation of reactions proceed-

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