# On evolutionary conservation of mitotic checkpoint signaling

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**The mitotic checkpoint is a control mechanism that ensures proper microtubule-kinetochore attachment and correct chromosome segregation during mitosis. A defective checkpoint signal can lead to chromosome missegregation and aneuploidy, which can lead to cancer. In this report I will summarize the different mitotic checkpoint proteins and their function in inhibiting metaphase to anaphase transition when defective kinetochore-microtubule attachment occurs. As research has been done in many different model organisms I will mainly focus on the evolutionary conservation of the mitotic checkpoint and its proteins. I conclude that the mitotic checkpoint complex is a well conserved mechanism from yeast to human. Although it seems that every organisms has some specific small differences and that higher eukaryotes need extra mechanisms to regulate the checkpoint, the core idea remains the same. Comparing the results obtained in different organisms also gives new insides in what features of the mitotic checkpoint are not yet well established and need more research.**

## Introduction

The process that separates the chromosomes of one nuclei from one eukaryotic cell into two nuclei is called mitosis (Figure 1). Cytokinesis divides the nuclei, cytoplasm, organelles and cell membrane into two separate cells and together with mitosis this process is called the mitotic (M) phase. The completion of one set of activities and the start of the next set determines specific phases in which mitosis is divided (Figure 1A). Correct chromosome segregation is essential for cells to maintain the integrity of their genome. Aneuploidy, an abnormal number of chromosomes, is known to result in severe birth defects like trisomies 13, 18 and 21. In addition, aneuploidy and chromosome instability (CIN), frequent loss and gain of chromosomes during cell division, are common aspects of human cancers (Lengauer et al., 1997; Weaver and Cleveland, 2006). Aneuploidy and CIN are probably caused by chromosome missegregation due to defects in mitotic control processes. One of these control processes is called the mitotic checkpoint which is activated during prometaphase to monitor proper microtubule-kinetochore attachment and correct chromosome segregation (Figure 1A). Mutations in mitotic checkpoint proteins have been shown to be present in various tumor samples, which indeed suggests that a failure in mitotic checkpoint activity is a cause for the aneuploidy observed in cancer (Figure 1B) (Cahill et al., 1998; Guo et al., 2010; Percy et al., 2000; Tsukasaki et al., 2001).

## Mitotic checkpoint basics

***Inhibition of the APC/C.*** The anaphase promoting complex/cyclosome (APC/C) is a multisubunit E3 ubiquitin ligase that controls mitotic progression and sister-chromatid segregation. Cell-division-cycle 20 homologue (CDC20) is an essential co-activator protein of the APC/C required for correct substrate recognition and interaction mediated by the presence of a KEN-box in these substrates (Peters, 2006; Pfleger and Kirschner, 2000). Two main substrates that are targeted for degradation by the APC/C and CDC20 are Cyclin B and Securin (Figure 2). Degradation of Cyclin B causes inactivation of Cyclin dependent kinase 1 (Cdk1), resulting in mitotic exit. Ubiquitination of Securin releases the inhibitory signal from Separase, which cleaves the cohesin rings surrounding the sister-chromatids, resulting in sister-chromatid segregation (Peters, 2006). In order to prevent the events of mitotic exit and sister-chromatid segregation when the chromosomes are not correctly attached to the microtubules, the mitotic checkpoint inhibits APC/C activity by targeting CDC20 (Cleveland et al., 2003). The first mitotic checkpoint proteins were identified by two independent genetic screens in *Saccharomyces cereviseae* (budding yeast) (Hoyt et al., 1991; Li and Murray, 1991). These proteins were named mitotic-arrest deficient (MAD) 1-3 and budding uninhibited by benzimidazole (BUB) 1-3. In a separate study also monopolar spindle (MPS) 1 was shown to be required for proper functioning of the mitotic checkpoint (Weiss and Winey, 1996). MAD2 and MAD3 (BUB1 related kinase (BUBR1) in higher eukaryotes) have been shown to be the checkpoint proteins responsible for the inhibition of the APC/C by their interaction with CDC20 (Li et al., 1997; Tang et al., 2001). In yeast, MAD2, BUBR1, BUB3 and CDC20 form the mitotic checkpoint complex (MCC) upon checkpoint activation (Hardwick et al., 2000). In vertebrates contradictory results were obtained concerning the formation of this MCC. Some suggest a yeast-like complex

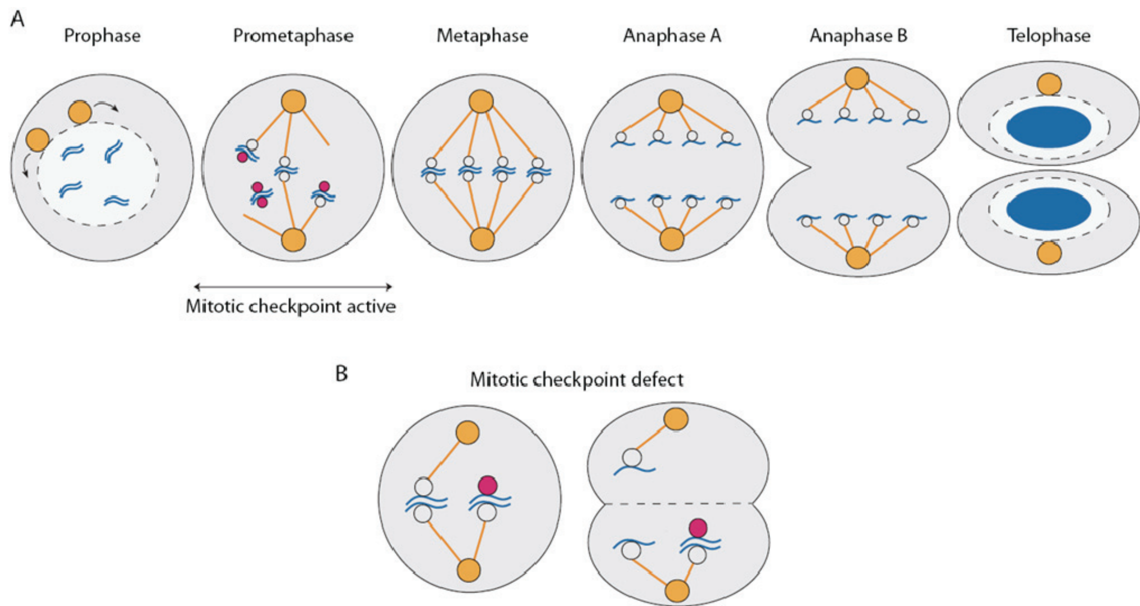


FIG. 1. (A) The six phases of mitosis. During prophase, condensation of the chromosomes occurs and the centrosomes (orange circles) separate to different poles. When nuclear envelope breakdown (NEB) occurs (dashed circle), the cell enters prometaphase. During this phase the chromosomes are localized to the cytoplasm and the microtubules from the centrosomes try to attached to the kinetochores (a multiprotein structure in the centre of a chromosome (centromere)). At unattached kinetochores (red dots) the mitotic checkpoint is activated. When both kinetochores of a chromosome pair are captured by microtubules (clear dots) the mitotic checkpoint is silenced and chromosomes are aligned to a midzone using microtubule motor activities and microtubule dynamics. Anaphase starts when all kinetochores are bound to microtubules, all chromosomes are aligned at the metaphase plate and all checkpoints are silenced. During anaphase A, each chromosome pair is separate to a different pole. In anaphase B, spindle elongation results in further chromosomes separation and invagination of the plasma membrane becomes visible. At the end of telophase the chromatin decondenses, the nuclear envelope is build up again and cytokinesis is completed. (B) Suggested involvement of the mitotic checkpoint in causing aneuploidy. A defective checkpoint fails to signal that one or more of the kinetochores are not captured by a microtubule. The weakened checkpoint is not able to generate a "wait anaphase" signal and the cell will go into anaphase, resulting in chromosome missegregation. Adapted from Kops et al, 2005.

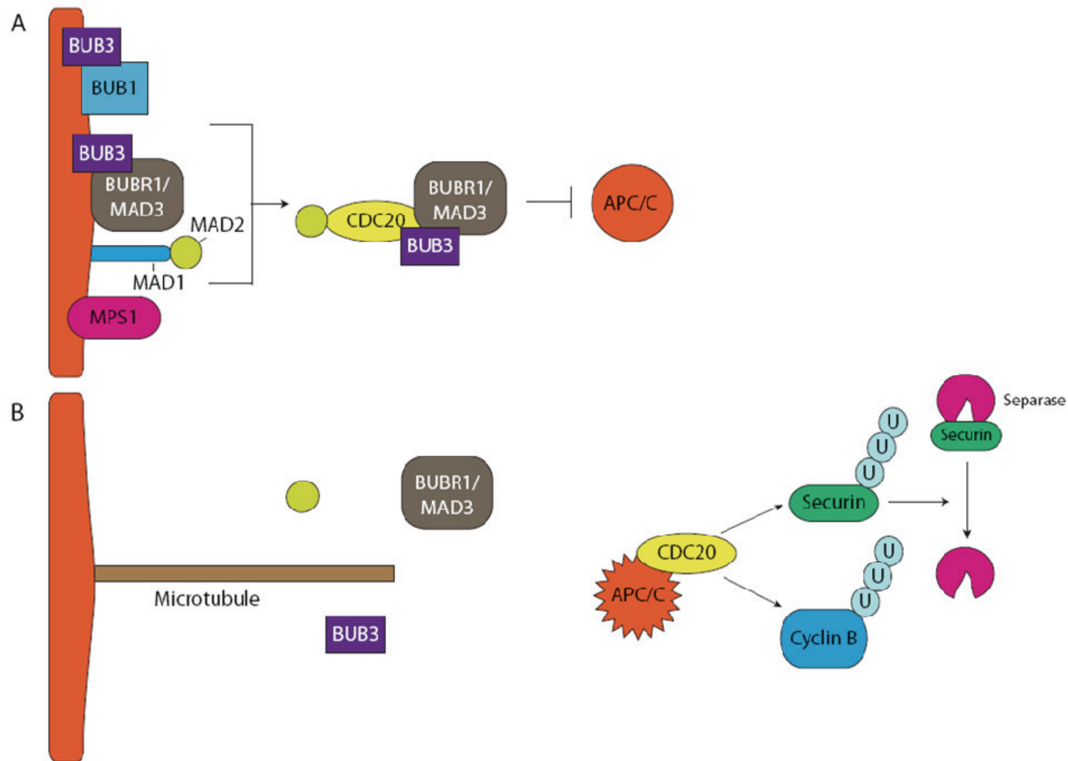


FIG. 2. Mitotic checkpoint and the APC/C controlling sister-chromatid segregation and mitotic progression. (A) Regulators MAD1, BUB1, BUB3 and MPS1 recruit the inhibitors MAD2 and BUBR1 to the kinetochore. BUB3, MAD2 and BUBR1 form the mitotic checkpoint complex (MCC) together with CDC20, which inhibits APC/C activation. (B) Upon attachment, the MCC falls apart and CDC20 interacts with the APC/C. Degradation of Securin releases inhibition of Separase. Separase cleaves the cohesin rings surrounding the sister-chromatids. Ubiquitination of Cyclin B causes inactivation of Cyclin dependent kinase 1 (CDK1) resulting in mitotic exit.

exist while others suggest BUBR1 and MAD2 form independent complexes with CDC20 (Fang, 2002; Herzog et al., 2009; Sudakin et al., 2001; Tang et al., 2001). The other proteins identified in the yeast screens MAD1, BUB1, BUB3 and MPS1, are necessary to regulated the formation of the mitotic checkpoint complex, mainly by recruiting the mitotic checkpoint complex proteins to the kinetochore (Figure 2) (Chen et al., 1999; Gillett et al., 2004; Maciejowski et al., 2010; Warren et al., 2002).

In this report I will summarize these different mitotic checkpoint proteins, the MCC and the regulators, and their function in inhibiting metaphase to anaphase transition when no or defective kinetochore-microtubule attachment occurs. As research has been done in many different model organisms I will mainly focus on the evolutionary conservation of the mitotic checkpoint and its proteins. Before going into this, a short introduction about the sensory machinery that is able to monitor incorrect microtubule attachment will be given.

***Sensory machinery.*** One single unattached kinetochore is able to inhibit the transition to anaphase. This suggests that the inhibitory signal needs to be amplified from one kinetochore to the whole cell (Rieder et al., 1995). Most mitotic checkpoint proteins have been shown to concentrate at unattached kinetochores during mitosis and to disappear from the kinetochores when microtubules are attached in a bi-orientated manner (Figure 3) (Chen et al., 1998; Chen et al., 1996; Li and Benezra, 1996; Taylor et al., 1998; Taylor and McKeon, 1997). Attachment of both sister kinetochores can also occur the wrong way (Figure 3). Due to a lack of tension, synthelic attachment leads to destabilization of microtubule kinetochore attachment by Aurora B kinase (Lens and Medema, 2003; Nicklas et al., 2001). In yeast, Aurora B seems required to activate the checkpoint in response to tension defects by generating unattached kinetochores (Figure 4) (Pinsky et al., 2006). This latter idea is supported by observations in human cells and rat kangaroo cells (Yang et al., 2009). Furthermore, an interaction between Aurora B and BUBR1/MAD3 has been shown in yeast and human cells, providing a link between Aurora B, unattached kinetochores and mitotic checkpoint activation (Figure 4) (King et al., 2007a; Morrow et al., 2005; Vanoosthuyse et al., 2009). Although it is suggest above that attachment is the only regulator of the activation or inactivation of the mitotic checkpoint, the idea of a tension "branch" and a attachment "branch" both capable of activation the checkpoint still exist. However, many contradictory results were obtained when trying the elucidate the role of a specific mitotic checkpoint protein in a specific branch. For example, the regulator BUB1 was suggested to be important for the tension "branch" in yeast,

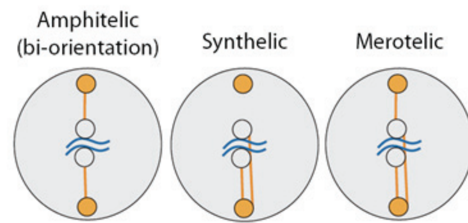


FIG. 3. Correct and incorrect attachment of both kinetochores. Amphitelic attachement is the correct attachment with the two sister kinetochores attached to different poles. In synthelic attachment, the two sister kinetochores are attached to one pole. In merotelic attachement one of the sister kinetochores is attached to both poles.

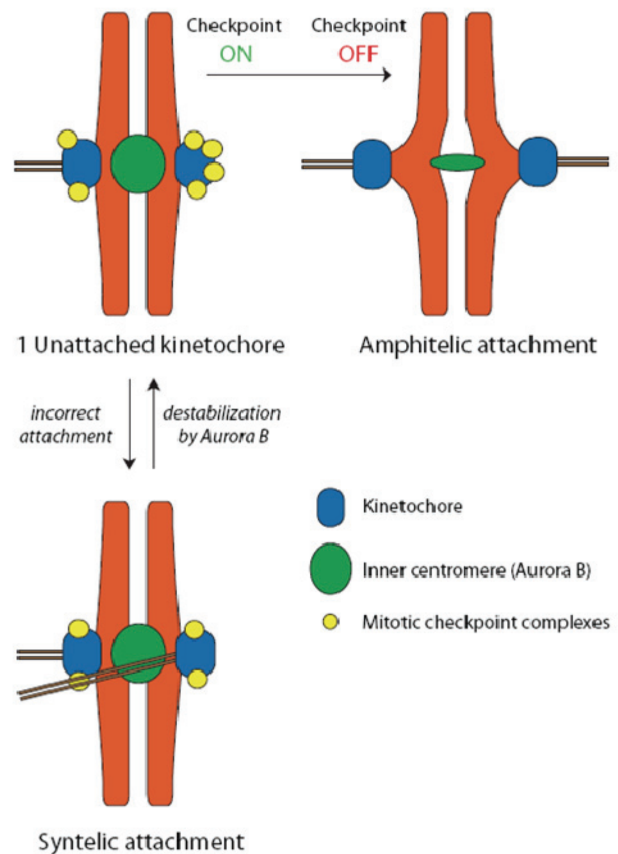


FIG. 4. Unattached kinetochore: The mitotic checkpoint complexes are recruited to unattached kinetochores and the checkpoint is active. Syntelic attachment: Aurora B destabilizes the incorrect microtubule attachment, resulting in unattached kinetochores again.. Amphitelic attachment: The kinetochores are physically displaced from the centromere and the checkpoint becomes silenced.

PtK1 cells and human cells, while in Drosophila BUB1 seemed more important in the attachment "branch" (Logarinho et al., 2004; Schliekelman et al., 2009; Shannon et al., 2002; Tournier et al., 2004). Another study suggested that BUB1 is needed in both branches (Taylor et al., 2001). These contradictory results also suggest that it is more likely that these two branches do not exist and that the checkpoint is only regulated by a lack of attachment.

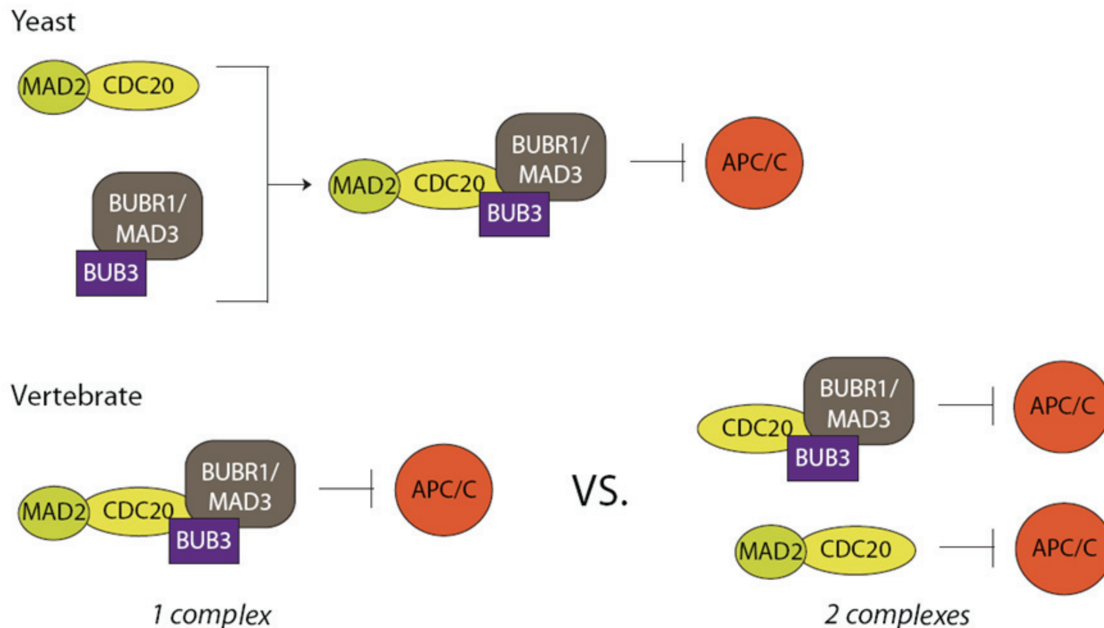


FIG. 5. MCC formation in yeast and vertebrates. Yeast: MAD2 (and possibly the interaction between MAD2 and CDC20) is required to form the MCC and inhibit the APC/C. Vertebrate: Conflicting data result in two different hypothesizes, the formation of 1 MCC as in the case in yeast or the formation of 2 complexes both capable of inhibiting the APC/C.

## The Mitotic Checkpoint Complex proteins

### Mitotic Checkpoint Complex formation

As will be discussed in more detail below, both BUBR1/MAD3 and MAD2 interact with CDC20 and inhibit APC/C activation. In yeast, *Drosophila*, *Xenopus* and human the formation of BUBR1/MAD3-MAD2 complex and the MAD2-CDC20 complex is upregulated during mitosis, which suggests a conserved mechanism (Chen, 2002; Fang et al., 1998; Fraschini et al., 2001; Hardwick et al., 2000; Li et al., 2010; Wu et al., 2000). However, different results were obtained on whether BUBR1/MAD3 and MAD2 form the mitotic checkpoint complex together with CDC20 and BUB3 and on how these two proteins are depending on each other in inhibiting the APC/C.

***MCC formation in yeast.*** In budding and fission yeast all four proteins form one complex, as was shown by co-immunoprecipitation and co-fractionation (Figure 5) (Fraschini et al., 2001; Hardwick et al., 2000; Millband and Hardwick, 2002). The formation of the CDC20-MAD2 is required for the interaction between MAD3 and CDC20, but not the other way around (Fraschini et al., 2001; Hardwick et al., 2000; Hwang et al., 1998; Poddar et al., 2005). In fission yeast, mitotic arrest upon overexpression of MAD2 requires MAD3, suggesting that both proteins are required for an efficient inhibition of the APC/C (Millband and Hardwick, 2002). The formation of the MCC is thought to be dependent on mitosis and not on activation of the mitotic checkpoint, as mitotic arrest with or without disturbing kinetochores interactions results in the same amount of MCC formed (Poddar et al., 2005). In addition, the formation of the MCC seems to be independent of the kinetochores because the MCC still forms when no intact kinetochores are present (Poddar et al., 2005).

***MCC formation in higher eukaryotes***. In human cells, contradictory results are obtained in different studies. First, the formation of a complex containing MAD2-CDC20-BUBR1-BUB3 was shown by fractionation and BUBR1 co-immunoprecipitation on a fraction containing the MCC (Figure 5) (Herzog et al., 2009; Sudakin et al., 2001), which is consistent with the results obtained in yeast. In contrast, in other studies no MAD2 was detected in a BUBR1 co-immunoprecipitation and vice-versa (Fang, 2002; Nilsson et al., 2008; Tang et al., 2001) suggesting no MCC, but two separate complexes are formed (Figure 5). Furthermore, Sudakin et al. suggested that the MCC is present throughout the cell cycle, but that only mitotic APC/C can be targeted. This observation is again in contrast with results obtained by others. In both human cells and *Xenopus* an interaction between CDC20 and BUBR1 and between CDC20 and MAD2 was detected only during mitosis and not only mitotic APC/C but also interphase APC/C could be inhibited (Chen, 2002; Fang, 2002). The discrepancies observed are possibly due to differences in experimental procedure and antibody use. Considering dependency of BUBR1 and MAD2 in their binding to CDC20 it seems to be the case (in human cells and *Xenopus*) that BUBR1 binding is indeed dependent on MAD2 expression, as is the case in yeast (Chen, 2002; Davenport et al., 2006; Fang, 2002). In contrast, *Drosophila* MAD2 is not required for a BUBR1-CDC20 interaction (Li et al., 2010). The other way

around, BUBR1 requirement for MAD2 to bind CDC20 is not that well established. A mild reduction in MAD2-CDC20 is seen in BUBR1 depleted *Xenopus* extracts, while in human cells only a role for BUBR1 could be detected when MAD2 and CDC20 were expressed at very low concentration (Chen, 2002; Fang, 2002). In this case the observation in *Drosophila* is more consistent compared to budding yeast, and no role for BUBR1 was detected in the formation of the CDC20-MAD2 complex (Li et al., 2010). Buffin et al. showed that flies without MAD2 are viable and fertile, which could be an explaining why BUBR1 is not depending on MAD2 in this organism (Buffin et al., 2007). Three separate studies in human cells showed that BUBR1 is a more potent inhibitor than MAD2. However, as is the case in fission yeast, maximal APC/C inhibition always requires both proteins, indicating the importance of the interaction/close proximity between BUBR1, MAD2 and CDC20 (Fang, 2002; Sudakin et al., 2001; Tang et al., 2001).

**MAD2 (-MAD1 complex)**

MAD2 is one of the inhibitory proteins in the MCC and forms a complex with MAD1 (Musacchio and Salmon, 2007). MAD2 homologues were usually identified by sequence homology. Human, *C. elegans* and *Xenopus* MAD2 all share around 40% identity with MAD2 from budding yeast and human and *Xenopus* MAD2 even share 80% identical amino acids (Chen et al., 1996; Kitagawa and Rose, 1999; Li and Benezra, 1996). Identification of MAD1 homologues was more difficult as the sequence is less conserved (around 20% comparing Human, *C. elegans* and *Xenopus* to budding yeast and 60% between *Xenopus* and human) and was usually identified as an interaction partner of MAD2 (Campbell et al., 2001; Chen et al., 1998; Kitagawa, 2009). Although the sequences were not well conserved, the predicted coiled-coil structures in all homologues were (Chen et al., 1998). Next to sequences and structures also protein functions are highly conserved

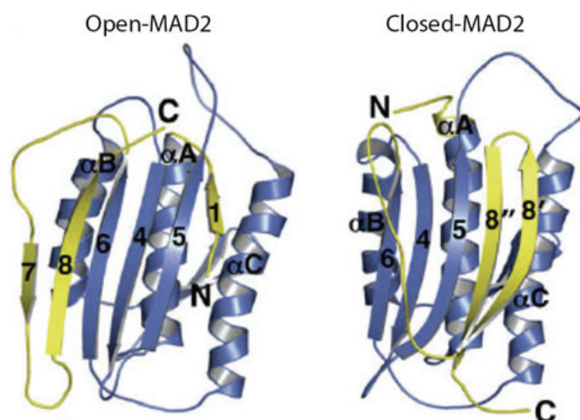


FIG. 6. Ribbon diagrams of the two states of MAD2, Open-MAD2 and closed MAD2. The C-terminal β-sheets (safety belt, yellow) are positioned in a different manner. Adapted from Luo et al, 2008

as both MAD1 (Chen et al., 1998; Encalada et al., 2005; Hardwick and Murray, 1995; Jin et al., 1998; Kitagawa and Rose, 1999; Luo et al., 2002) and MAD2 (Buffin et al., 2007; Chen et al., 1996; Dobles et al., 2000; Gorbsky et al., 1998; Hoyt et al., 1991; Kitagawa, 2009; Li and Murray, 1991; Li and Benezra, 1996) are required in all organisms for proper functioning of the mitotic checkpoint after addition of spindle poisons. Interestingly, MAD2 depletion only resulted in loss of viability in higher eukaryotes, indicating a role for the checkpoint during normal cell division in these organisms (Chen et al., 1996; Dobles et al., 2000; Gorbsky et al., 1998; Li and Benezra, 1996). Consistent with this observation MAD2 and MAD1 were localized to the kinetochores during prometaphase in normally dividing mammalian cells (Campbell et al., 2001; Gillett et al., 2004). MAD2 in yeast is not essential for normal cell division and indeed MAD1 and MAD2 were only detected at the kinetochores of cells with disrupted microtubule-kinetochore interactions (Li and Murray, 1991). Also in *C. elegans* MAD2 was localized to the holocentric-kinetochores only when cells were treated with nocodazole and MAD2 seemed not needed for viability (Kitagawa and Rose, 1999). Interestingly, *Drosophila* MAD2 was also indicated not to be required for viability, but in contrast to yeast and *C. elegans*, *Drosophila* MAD2 does localize to the kinetochores during normal cell division (Buffin et al., 2007; Logarinho et al., 2004). This could be explained by the fact that *Drosophila* has a very efficient mitosis and that the spindle microtubules and the kinetochores attach so rapidly that MAD2 is not yet required (but still localized). This explanation is in support with the observation that the prometaphase in *Drosophila* takes only 5 minutes, while it takes 15-20 minutes or more in vertebrate cells (Meraldi et al., 2004; Savoian and Rieder, 2002; Siller et al., 2005).

***MAD1-MAD2 kinetochore recruitment and dynamics.***

As mentioned, a conserved interaction is present between MAD1 and MAD2. This interaction is present throughout the cell cycle and required for the kinetochore localization of MAD2 (Chen et al., 1999; Chen et al., 1998; Chung and Chen, 2002; De Antoni et al., 2005; Howell et al., 2004; Luo et al., 2002; Vink et al., 2006). When bound to MAD1, MAD2 adopts a typical conformation, known as closed-MAD2 (C-MAD2) (Luo et al., 2002; Sironi et al., 2002). Although many studies focus on mammalian cells and little on *Xenopus*, *Drosophila* and yeast, the fact that the coiled-coil domains of MAD1 are conserved and the fact that these domains seem to be important for the MAD2 conformational change, suggests conservation (Sironi et al., 2002). The MAD1-C-MAD2 complex is very stable and results in a stable C-MAD2 kinetochore pool (De Antoni et al., 2005; Howell et al., 2004; Shah et al., 2004; Vink et al., 2006). MAD1-C-MAD2 is a receptor required to attract

CDC20

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Hs  124  DVEEAKILRLSGKP   137
Dm  151  DSKGGRILCYQNKAP  165
Sc  197  DMN-KRILQYMPEPP  210
Sp  124  DLN-TRVLAFKLDAP  137
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MAD2-binding domain

MAD1

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Hs  540  TKVLHMSLNPTS  551
Dm  551  FKVVHFSENPAA  562
Sc  580  IRILQLRDGPFI  591
Sp  522  CRVLQHRSNPTL  533
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MAD2-binding domain

FIG. 7. Alignment of the MAD2 binding sequences (red) of both CDC20 and MAD1 from different species. Sc, *S. cerevisiae*; Sp, *S. pombe*; Dm, *Drosophila melanogaster;* Hs, *Homo sapiens.* Adapted from Luo et al, 2002.

another pool of MAD2 to the kinetochore, termed open-MAD2 (O-MAD2) (De Antoni et al., 2005; Luo et al., 2000; Luo et al., 2002; Sironi et al., 2002; Vink et al., 2006). The two MAD2 conformations differ in the position of the C-terminal tail, which is composed of two β-sheets and is also called "the safety belt" (Figure 6). In the closed conformation this tail traps the MAD2 interaction partners, while in the open conformation the tail is in a "resting" position (Luo et al., 2002; Sironi et al., 2002). The idea of MAD1-C-MAD2 being a receptor for O-MAD2 is supported by the fact that C-MAD2 and O-MAD2 have been shown to form a asymmetric dimer and by the fact that mutations (in mammalian cells, yeast and *Drosophila*) disrupting this dimerization result in a loss of the mitotic checkpoint (De Antoni et al., 2005; Li et al., 2010; Mapelli et al., 2006; Mapelli et al., 2007; Nezi et al., 2006; Yang et al., 2008).

***The interaction with CDC20.*** Next to the interaction with MAD1, MAD2 also interacts with CDC20. This interaction is conserved in yeast, *Xenopus* and humans and results in inhibition of the APC/C (Fang et al., 1998; Hwang et al., 1998; Kim et al., 1998; Li et al., 1997). CDC20 triggers the same conformational change of MAD2 as MAD1, from O-MAD2 to C-MAD2. This almost identical conformation change is due to the fact that MAD1 and CDC20 have similar MAD2-binding domains (Figure 7) (Luo et al., 2002; Luo et al., 2004; Sironi et al., 2002). The sequences of these binding domains are not strictly conserved during evolution, but aromatic residues (Tyr (Y), Phe (F), or His (H)) in the binding sequence and a proline C-terminal of the binding motif can be observed in most of the organisms, possibly forming a consensus sequence (Luo et al., 2002). The formation of the CDC20-C-MAD2 complex is increased during mitosis and the interactions is required for activation of the mitotic checkpoint, as mutations in the MAD2 binding site of CDC20 resulted in defective checkpoint signaling (Fang et al., 1998; Kallio et al., 1998; Wassmann and Benezra, 1998). The ideas on how MAD2 is able to activate the checkpoint form the "template model" (Figure 8). This model proposes that a stable MAD1-C-MAD2 complex recruits O-MAD2 to the unattached kinetochores. The binding of O-MAD2 to C-MAD2 is required for CDC20 to bind to the O-MAD2 and trigger the conformational change, resulting in CDC20-C-MAD2. (Simonetta et al., 2009; Yang et al., 2008). Overexpression of MAD1 in cells and *Xenopus* extracts inactivated the checkpoint, possibly by altering the balance between MAD1 bound MAD2 and CDC20 bound MAD2 (Canman et al., 2002; Chung and Chen, 2002). It is though that not only MAD1-C-MAD2 triggers binding of MAD2 to CDC20 but also CDC20-C-MAD2 itself, resulting in a positive feedback loop (Figure 8). This loop would explain how one unattached kinetochore is able to inhibit all APC-CDC20 in a whole cell. In yeast, MAD1, MAD2 and CDC20 were found in association with each other, while this is not the case in human cells and not completely in line with the model proposed (Campbell et al., 2001; Hwang et al., 1998). As the interaction observed between MAD1 and CDC20 was very low, possibly MAD1 was immunoprecipitated because of the interaction with the C-MAD2-O-MAD2 dimer (Hwang et al., 1998).

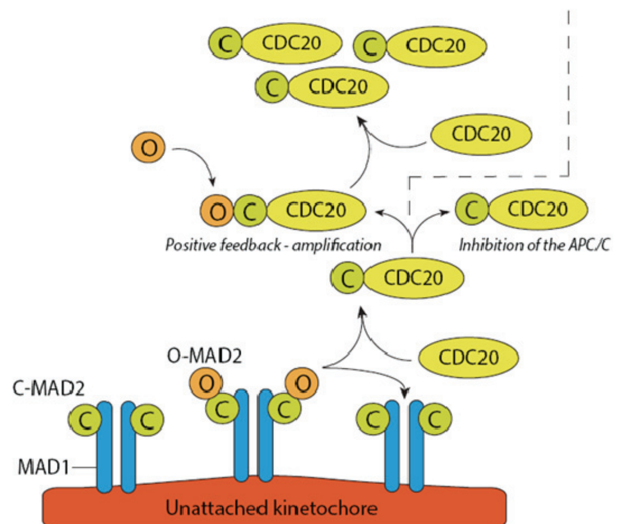


FIG. 8. The MAD2 "template model". Unattached kinetochores recruit the MAD1-C-MAD2 complex. This "template" recruits O-MAD2 to the kinetochores to facilitate the binding with CDC20. Upon binding with CDC20, O-MAD2 becomes C-MAD2, resulting in inhibition of the APC. CDC20-C-MAD2 is possibly also involved in a positive feedback loop, generation amplification of the signal. Adapted from Musacchio and Salmon, 2007.

In summary, both MAD1 and MAD2 are required for the activation of the mitotic checkpoint. C-MAD2 interacts with CDC20 to inhibit the APC/C and MAD1 is needed to localize MAD2 to the kinetochore and to change O-MAD2 into C-MAD2. Although most assays concerning the conformational changes of MAD2 were done in vitro or in higher eukaryotes, the similarity in sequences, interaction patterns and function of MAD1, MAD2 and CDC20 indicate that this mechanism is highly conserved.

**BUBR1/MAD3**

The other inhibitory protein in the MCC is BUBR1. BUBR1 is the homologue of the MAD3 protein originally identified in yeast (Figure 9). Both BUBR1 and MAD3 have a homologues domain with BUB1. It is believed that MAD3 origin lays in a ancient whole-genome duplication in fungi, resulting in a duplication of BUB1 (Kellis et al., 2004; Larsen et al., 2007). The N-terminal part of both BUBR1 and MAD3 contain two conserved KEN-boxes and the C-terminal part of BUBR1 contains a kinase domain. This kinase domain is not present in MAD3. As BUBR1 retained its kinase domain it is thought that BUBR1 arose from a separate whole-genome duplication event (Larsen et al., 2007; Larsen and Harrison, 2004). The role of the KEN-boxes and the kinase domain will be discussed below. In all organisms studied is has been shown that MAD3/BUBR1 is important to generate an active mitotic checkpoint when microtubule-kinetochore attachment is abolished by spindle poisons. (Chan et al., 1999; Chen, 2004; Hoyt et al., 1991; Li and Murray, 1991; Moore et al., 2005; Nystul et al., 2003; Rahmani et al., 2009). In *C. elegans* and yeast, MAD3 is not required for proper chromosome segregation during normal cell division (Buffin et al., 2007; Stein et al., 2007; Warren et al., 2002). As this was also the case for MAD2 in both organisms it seems that the mitotic checkpoint is dispensable for viability in these organisms. In higher eukaryotes, BUBR1 has been shown to be necessary for correct timing between nuclear envelop breakdown (NEB) during normal cell division, but this role of BUBR1 seems to be independent of its role in the mitotic checkpoint (Meraldi et al., 2004; Rahmani et al., 2009).

***Interaction with BUB3.*** BUB3 is another checkpoint protein identified by the yeast genetic screens that is conserved throughout evolution (Campbell and Hardwick, 2003; Larsen and Harrison, 2004). Initially an interaction between BUB1 and BUB3 was identified (see below). After the whole genome duplication the N-termini of BUB1 and BUBR1/MAD3 remained the same. As the BUB3 binding domain of BUB1 is located in this N-terminus, an interaction between BUB3 and BUBR1/MAD3 was expected. Indeed, next to BUB1 also BUBR1/MAD3 interacts with BUB3 through its conserved BUB3 binding region (Figure 4) (Musacchio and Salmon, 2007). Both the BUB1-BUB3 and BUBR1-BUB3 complex have been shown to be present throughout the whole cell cycle in human cells, yeast and *Xenopus* (Chen, 2002; Hardwick et al., 2000; Taylor et al., 1998). The interaction with BUB3 seems to be important for BUBR1/MAD3 localization to the kinetochore (Gillett et al., 2004; Harris et al., 2005; Taylor and McKeon, 1997). However, BUB3 is not necessary for the inhibition of the APC/C through the interaction with CDC20 (Fang, 2002; Tang et al., 2001) This suggests, as will be discussed in more detail below, a role for BUB3 in kinetochore localization and possibly in generating a more efficient checkpoint, but not in direct inhibition of the APC/C.

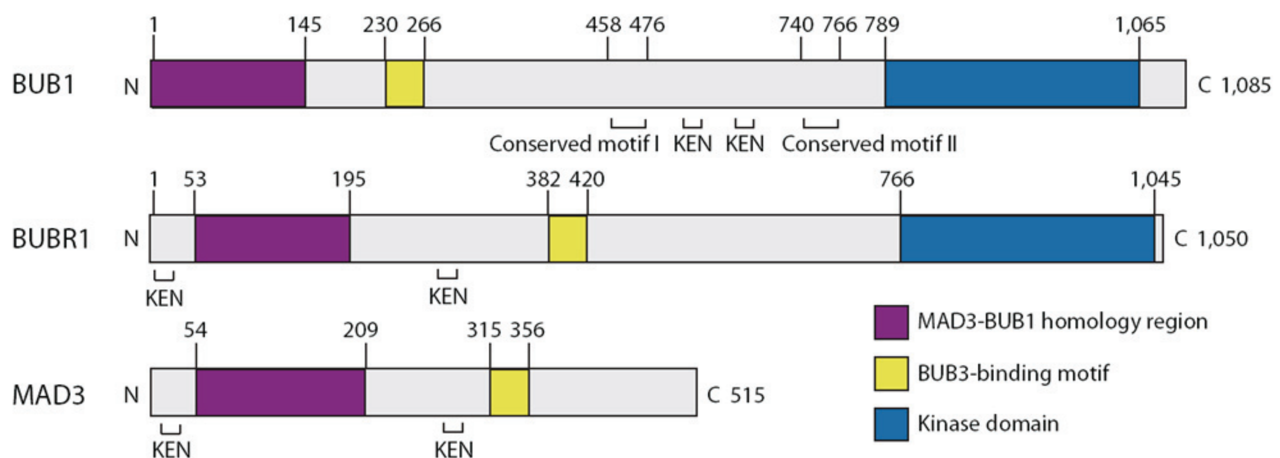


FIG. 9. Schematic view of human BUB1, human BUBR1 and yeast MAD3. Human BUBR1 and yeast MAD3 are homologues and are proteins related to BUB1. BUBR1 and MAD3 both have a BUB3 binding domain and a KEN-box. In addition, BUBR1 contains a C-terminal kinase domain which is not present in MAD3. BUB1, BUBR1 and MAD3 all contain a BUB1-MAD3 homology domain. Next to these domains, BUB1 contains two motifs that are highly conserved throughout evolution. Adapted from Musacchio and Salmon, 2007.

FIG. 10. Alignment of the KEN-boxes of MAD3/BUBR1 proteins from various species. Completely conserved residues are labeled in black and highly conserved residues in gray. Sequences were aligned using the Clustal W program. Adapted from Burton and Solomon, 2007.

***Inhibition of the APC/C through CDC20.*** In yeast and human cells, both BUBR1 and MAD3 have been shown to interact with CDC20, resulting in inhibition of the APC/C (Hardwick et al., 2000; Tang et al., 2001). Furthermore, it was shown in mice that BUBR1 acts as a pseudosubstrate inhibitor for the APC/C, as the APC/C regulated Cyclin B degradation is much higher when the N-terminus of BUBR1 is mutated (Malureanu et al., 2009). Two KEN-boxes were shown to be highly conserved in BUBR1/MAD3 (Figure 10). Mutating these KEN-boxes one by one revealed that the N-terminal KEN-box is required for CDC20 interaction in all organisms studied, budding yeast, fission yeast, *Drosophila*, mice and human (Burton and Solomon, 2007; Elowe et al., 2010; King et al., 2007b; Malureanu et al., 2009; Rahmani et al., 2009; Sczaniecka et al., 2008). The role of the second KEN-box is less clear. In mice studies both KEN-boxes were important for CDC20 binding (Malureanu et al., 2009), while in yeast and human cells it was shown that the second KEN-box is not as important (Elowe et al., 2010; King et al., 2007b). Interestingly, the second KEN-box is not present in *Drosophila* (Figure 10), suggesting that the role of the second KEN-box is not very important or that *Drosophila* developed a feature to compensate for missing that box. It was suggested that the C-terminus in general of BUBR1 also plays a role in CDC20 binding (Elowe et al., 2010). It is possible that mutating the second KEN-box in mice abolished the structure of the whole C-terminus resulting in a (KEN-box independent) decreased CDC20 interaction. This explanation could explain the contradictory result and suggests that the second KEN-box is indeed less important for CDC20 binding. Another role for the second KEN-box in correct docking and orientation of BUBR1 to the APC/C has been proposed, but more research is needed to support this idea (Elowe et al., 2010).

***Kinase activity of BUBR1 and the mitotic checkpoint.*** As the C-terminal kinase domain is not conserved between lower eukaryotes (yeast and *C. elegans*) and higher eukaryotes (*Drosophila*, *Xenopus* and humans) its role is studied extensively. So far, some conflicting data have been obtained concerning the influence of BUBR1 kinase activity on the mitotic checkpoint. BUBR1 proteins with a mutated or deleted kinases domain were still capable to inhibit the APC/C (shown in both human cells and *Xenopus* extracts) (Chen, 2002; Tang et al., 2001). In contrast, in both *Xenopus* abstract and human tumors a role for BUBR1 kinase activity was suggested to be important for the checkpoint (Kops et al., 2004; Mao et al., 2003), but this latter two studies did not assay directly on APC/C activity. A third observation suggested a role for BUBR1 kinase activity in the long-term maintenance of a nocodazole-induced mitotic arrest. In human cells, mice and *Drosophila* absence of BUBR1 kinase activity could restore mitotic progression, but no pronounced delay could be observed (Huang et al., 2008; Malureanu et al., 2009; Rahmani et al., 2009). Whether this loss of maintenance is caused by a weaker checkpoint, possibly by a loss in efficiency, or by mitotic checkpoint unrelated BUBR1 characteristics is not known. Since Yeast and *C. elegans* do not contain the kinase domain the most logical explanation would be that the kinase domain is not involved in the core of mitotic checkpoint, as these organism still can "do the job". In yeast the microtubules are always bound the kinetochores and a disturbed cell division timing in *C. elegans* results in developmental defects (Gillett et al., 2004; Kitagawa, 2009). This suggest that these two organisms possibly do not need the maintenance of the mitotic arrest and in that case not need the kinase domain.

***CENP-E and BUBR1.*** CENP-E is a microtubule plus-end-directed motor, that interacts with, get phosphorylated by and activates BUBR1 (Chan et al., 1999; Chan et al., 1998; Mao et al., 2003; Mao et al., 2005; Yao et al., 2000). In *Xenopus*, the activation of BUBR1 seems to be dependent on microtubule binding of CENP-E, as CENP-E-microtubule interaction inhibits BUBR1 activity (Mao et al., 2003; Mao et al., 2005). Furthermore, again demonstrated in *Xenopus*, CENP-E deletion results in a loss of mitotic checkpoint signaling, suggesting CENP-E is a component of the checkpoint (Abrieu et al., 2000). In contrast, studies in *Drosophila*, human cells and mice cells demonstrated that CENP-E is not needed for mitotic checkpoint signaling (Jeganathan et al., 2007; Maia et al., 2007; McEwen et al., 2001; Tanudji et al., 2004; Yao et al., 2000). These results and the fact that CENP-E is not conserved in yeast and *C. elegans*, suggests that CENP-E (and the interaction with BUBR1) is not an essential component of the mitotic checkpoint.

***Other roles of BUBR1/MAD3.*** Next to its role in the mitotic checkpoint (and mitotic timing), BUBR1 is required for the establishment of stable interaction between the kinetochore and spindle microtubules (Ditchfield et al., 2003; Lampson and Kapoor, 2005). The kinase activity, that was not important for the mitotic checkpoint, seems to play a role in the establishment of these correct microtubule-kinetochore attachment in *Drosophila*, *Xenopus* and human (Huang et al., 2008; Rahmani et al., 2009; Zhang et al., 2007). So far it has been suggested that this role of BUBR1 is mediated by counteracting with Aurora B activity and recruitment of APC-EB1 (Kang and Yu, 2009). Interestingly, Maia et al. suggests that CENP-E possibly also acts in promoting the correct microtubule-kinetochore attachment by a counteracting mechanism like Aurora B (Maia et al., 2007). This suggestion still links the kinase activity of BUBR1 to CENP-E, as observed in Xenopus, and is consistent with the fact that two organisms (yeast and *C. elegans)* in which MAD3 is lacking the kinase domain, also lack CENP-E. This latter idea suggests that yeast and *C. elegans* cannot correct microtubule-kinetochore attachment. Yeast indeed do not need this correction as microtubules are always bound the kinetochores, and only 1 microtubule binds to 1 kinetochore (Gillett et al., 2004). In *C. elegans*, the facts that only 2x6 chromosomes are present and that timing is crucial for development could suggest that this correction is also not needed in this organism (Kitagawa, 2009).

In short, BUBR1/MAD3 is required for proper mitotic checkpoint signaling. The interaction with CDC20 through the N-terminal KEN-box results in APC/C inhibition and the interaction with BUB3 is most likely required to generate a more efficient checkpoint. The exact role of the BUBR1 kinase domain still needs to be determined, but it is possibly involved in the long-term maintenance of a mitotic arrest. Whether this latter function is regulated by mitotic checkpoint related or unrelated BUBR1 characteristics is not known.

## The mitotic checkpoint regulators

### BUB1

BUB1 is a highly conserved mitotic checkpoint protein, and BUB1 inactivation or depletion results in mitotic checkpoint loss in fission yeast, budding yeast, *C. elegans*, *Drosophila*, *Xenopus*, mice and humans (Basu et al., 1999; Bernard et al., 1998; Chen, 2004; Encalada et al., 2005; Farr and Hoyt, 1998; Logarinho et al., 2004; Meraldi and Sorger, 2005; Perera et al., 2007; Tang et al., 2004a; Warren et al., 2002). BUB1 is a protein kinase that contains a BUB3-binding domain and a kinase domain (Figure 9). In yeast, *Xenopus* and human it has been shown by a Δ*bub1* strain, BUB1 immunodepletion or BUB1 RNAi that BUB1 is required for the recruitment of the checkpoint proteins MAD1, MAD2 and BUBR1/MAD3 to the kinetochores (Gillett et al., 2004; Johnson et al., 2004; Meraldi et al., 2004; Sharp-Baker and Chen, 2001).

***Interaction with BUB3.*** The interaction between BUB1 and BUB3 is highly conserved, and loss of the BUB3 binding domain or loss of BUB3 in general results in insufficient BUB1 kinetochore localization (in budding yeast, fission yeast and human cells) (Kitagawa, 2009; Klebig et al., 2009; Roberts et al., 1994; Warren et al., 2002; Windecker et al., 2009). In *Xenopus*, antibodies blocking BUB3 function could not prevent BUB1 or BUB3 kinetochore localization, but this contradictory result could be due to the antibodies used (Campbell and Hardwick, 2003). On the exact role of BUB3 (and BUB1 kinetochore localization) in the mitotic checkpoint different results were obtained. In budding yeast, C. *elegans*, *Xenopus* and human cells BUB3 has been shown to be essential for mitotic checkpoint signaling (Campbell and Hardwick, 2003; Hoyt et al., 1991; Kitagawa, 2009; Li and Murray, 1991; Logarinho et al., 2008). In fission yeast however, BUB3 is not a core component of the spindle checkpoint, as the checkpoint is still partially active in ΔBUB3 cells (Vanoosthuyse et al., 2009). Reports, in fission yeast, mice, human and *C. elegans* suggest that BUB1 and other

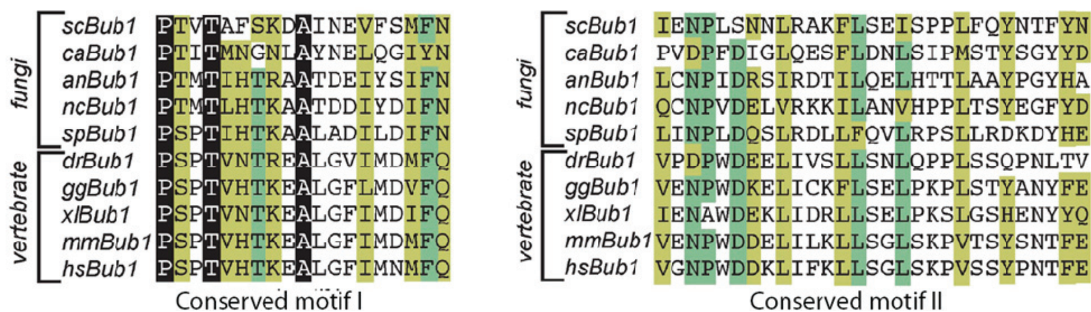


FIG. 11. Aligned sequences of the two conserved motifs of BUB1 in five fungi and five vertebrates. Identical residues are in a black, residues conserved in ≥80% of the species are in a dark green, and similar residues in ≥80% of the species are in a light green background. Sc, *S. cerevisiae*; ca, *Candida albicans*; an, *Aspergillus nidulans*; nc, *Neurospora crassa*; sp, *S. pombe*; dr, *Danio rerio*; gg, *Gallus gallus*; xl, *X. laevis*; mm, *Mus musculus*; hs, *Homo sapiens*. Adapted from Klebig et al, 2009.

checkpoint proteins (MAD2 and MAD3/BUBR1) can still (partially) function independent of kinetochore localization (Essex et al., 2009; Klebig et al., 2009; Malureanu et al., 2009; Windecker et al., 2009) In human cells and budding yeast, BUB1 lacking the BUB3 binding domain was not capable of activating the checkpoint, leading to the conclusion that the kinetochore localization and interaction with BUB3 was essential for the checkpoint (Klebig et al., 2009; Warren et al., 2002). However, it could also be the case that these mutants were not only defective in BUB3 binding but also in general BUB1 function, suggesting BUB1 localization by BUB3 is indeed not essential for the checkpoint. These observations led to the hypothesis that BUB3 is needed for the localization of BUB1 and other checkpoint proteins making the checkpoint more efficient and that it is possible that BUB3 fulfills this similar role in the checkpoint of all eukaryotes. Whether this BUB3 localization function is essential for mitotic checkpoint activity remains to be elucidated, as it seems to be in most but not all eukaryotes (fission yeast).

***BUB1 kinase activity.*** Some controversial results were obtained focusing on the role of the evolutionary conserved kinase domain of BUB1 (Tang et al., 2004a). In budding yeast and mice cells, loss of BUB1 kinase activity did not perturb the checkpoint at all (Cowley et al., 2005; Fernius and Hardwick, 2007; Roberts et al., 1994; Warren et al., 2002). In fission yeast, *Xenopus* or human cells loss of kinase activity did result in a partial rescue of the mitotic checkpoint (Chen, 2004; Klebig et al., 2009; Yamaguchi et al., 2003). Interestingly in *Xenopus*, they showed that when treating the cells in such a way that all attachments of microtubules to kinetochores are disrupted, loss of kinase activity was not sufficient to rescue the checkpoint, suggesting a role for BUB1 kinase activity in the efficiency of the checkpoint (Chen, 2004). It is possible that this role in efficiency and the use of different poisons resulted in the complete or partial rescues observed in the different organisms. Furthermore, in human, BUB1 has been shown to be responsible for the phosphorylation and inhibition of CDC20 mediated by direct binding to two KEN-boxes (Figure 9) (Kang et al., 2008; Tang et al., 2004a). Both the KEN-boxes and the phosphorylation of CDC20 have been shown to be important but not essential in mitotic signaling. Interestingly both the KEN-boxes and the BUB1 phosphorylation sites on CDC20 are not conserved throughout evolution, which suggests that BUB1 mediated CDC20 phosphorylation is an extra tool for CDC20 inhibition in higher eukaryotes (Qi and Yu, 2007; Vanoosthuyse and Hardwick, 2005). All together these results suggest that the BUB1 kinase domain is not essential for the activation of the mitotic checkpoint, but more likely for the efficiency of the checkpoint, and for chromosome congression (see below).

***Conserved motifs I & II*** Next to the BUB3 binding domain and the kinase domain Klebig et al. identified two conserved motifs present in budding yeast, fission yeast, zebrafish, chick, *Xenopus*, mice and human (Figure 10 and 11) (Klebig et al., 2009). Cells expressing BUB1 without conserved motif I failed to activate the mitotic checkpoint, but did show normal chromosome alignment. In contrast the conserved motif II appeared to be required for both the spindle checkpoint and for chromosome alignment. Furthermore, a deletion of this domain resulted in a reduction of localization of other mitotic checkpoint to the kinetochores (Klebig et al., 2009). Interestingly the separate roles of two BUB1 domains, chromosomal congression (kinases domain) and the mitotic checkpoint (conserved motif I) was also observed in yeast, which suggests a conserved mechanism (Warren et al., 2002)

***Other BUB1 functions*.** Activation of mitotic checkpoint signaling is not the only role for BUB1 during mitosis. In animal cells (and *in vitro* in yeast) the kinetochores first bind to the sides of the microtubules after which a correction-mechanism results in end-on attachment (Merdes and De Mey, 1990; Sorger et al., 1994). Depletion of BUB1 results in a delayed formation of stable end-on attachments, leading to an accumulation of lateral kinetochore–microtubule attachments. This indicates a conserved role for BUB1 is correcting kinetochore-"lateral"-microtubule attachment (Gillett et al., 2004; Meraldi and Sorger, 2005; Williams et al., 2007). Furthermore it has been shown that BUB1 is important in the correction of chromosome congression (Johnson et al., 2004; Meraldi and Sorger, 2005). Both the conserved motif II and the highly conserved kinase domain have been demonstrated to be important for the congression of the chromosomes (Klebig et al., 2009). Shugoshin is a key target of BUB1 in chromosomal alignment and from yeast to human it has been shown that Shugoshin is required for correct chromosomal alignment (Katis et al., 2004; Kerrebrock et al., 1995; Kitajima et al., 2005; Kitajima et al., 2004; Rabitsch et al., 2004; Salic et al., 2004; Tang et al., 2006; Tang et al., 2004b). Kawashima et al. showed in fission yeast that BUB1 phosphorylates histone H2A, providing a mark for Shugoshin localization (Kawashima et al., 2010). It has been proposed that BUB1 fulfills both roles, correcting kinetochore-"lateral"-microtubule attachment and chromosome congression, together with BUB3 (Logarinho et al., 2008; Windecker et al., 2009) In order to activate substrates, BUB1 needs to be activated itself. BUB1 is able to autophosphorylate and it is hyperphosphorylated in a Mitogen Activated Protein Kinase (MAPK) dependent manner at unattached kinetochores in *Xenopus* (Chen, 2004; Roberts et al., 1994). Although it is not know whether this hyperphosphorylation also occurs in yeast, the activation by MAPK seems to be a conserved mechanism as CDC2 (in

yeast) and MAPK (in *Xenopus*) have similar consensus sequences. Furthermore sequence alignment of the putative MAPK residue in *Xenopus* BUB1 corresponded to a residue found in fission yeast BUB1 (Chen, 2004).
Briefly, BUB1 is a conserved protein essential for mitotic checkpoint signaling. The function of the conserved motif I and II is most likely conserved from yeast to human. Also the interaction with BUB3 is present in all eukaryotes studied, but the requirement of BUB3 remains controversial and possibly species specific. Furthermore, the conserved BUB1 kinase domain seems not essential for the activation of the mitotic checkpoint, but more likely for the efficiency of the checkpoint (by CDC20 phosphorylation in higher eukaryotes).

**MPS1**

MPS1 is a dual specificity kinase, initially discovered to regulated spindle pole body duplication in budding yeast (Winey et al., 1991). The MPS1 protein is conserved throughout evolution and was originally identified as TTK in humans, as Esk in mice, as Ald in *Drosophila* and as MPS1 in *Xenopus* (Abrieu et al., 2001; Douville et al., 1992; Gilliland et al., 2005; Mills et al., 1992). Later, the role of MPS1 in the mitotic checkpoint was discovered in budding yeast (Weiss and Winey, 1996). This role of MPS1 is evolutionary conserved in fission yeast, *Drosophila*, zebrafish, *Xenopus* and human. (Abrieu et al., 2001; Fischer et al., 2004; He et al., 1998; Liu et al., 2003; Poss et al., 2002). MPS1 has been shown to be localized to the kinetochores in the period from NEB to the start of metaphase, referring to a period with unattached kinetochores, in *Drosophila*, *Xenopus* and human cells (Abrieu et al., 2001; Fischer et al., 2004; Liu et al., 2003). MPS1 localization to the kinetochores is also detected after checkpoint activation (Stucke et al., 2004; Stucke et al., 2002).

***Function of MPS1 in mitotic checkpoint signaling***. In contrast to the fact that the importance of MPS1 in the mitotic checkpoint is clear and conserved, the exact role of MPS1 in the checkpoint is less obvious. In human cells, depletion of MPS1 through RNAi and inhibition of MPS1 kinase activity resulted in a loss of only MAD2 at the kinetochores (Jelluma et al., 2008; Liu et al., 2003; Stucke et al., 2002; Tighe et al., 2008). In human cells with depleted MPS1 via gene-targeting or cells stably expressing MPS1 without kinase activity, loss of all checkpoint proteins at the kinetochore could be seen (Maciejowski et al., 2010; Sliedrecht et al., 2010). Discrepancies are most likely due to incomplete RNAi knockdown in the earlier studies as the latter observations are similar to results obtained in *Xenopus* and yeast (Abrieu et al., 2001; Hardwick et al., 1996; Vigneron et al., 2004; Wong and Fang, 2005). In yeast and *Drosophila*, MPS1 has been shown to interact with both MAD1 and MAD2. Although this interaction is possibly indirect, it confirms the idea that MPS1 recruits MAD1 and MAD2 to the kinetochore (Lince-Faria et al., 2009). Another aspect of MPS1 conserved in yeast and human is the fact that, apart from MAD1, MPS1 is the only other checkpoint factor required for the interaction between CDC20-MAD2 and CDC20-BUBR1 (Hwang et al., 1998; Maciejowski et al., 2010; Sliedrecht et al., 2010). The kinase activity of MPS1 seems to be important for auto-phosphorylation and MPS1 recruitment to the kinetochores (Xu et al., 2009). Consistent with this idea, MPS1 is indeed hyperphosphorylated during mitosis (Fisk et al., 2004; Liu et al., 2003; Stucke et al., 2004; Stucke et al., 2002). However, this hyperphosphorylation is possibly also regulated by other upstream kinases. Yeast MPS1 kinase activity induces mitosis specific MAD1 phosphorylation (Hardwick and Murray, 1995; Hardwick et al., 1996), but this observation has never been reported for human MAD1 (Campbell et al., 2001). Whether the role of MPS1 to phosphorylate MAD1 is conserved needs to be further established by the use of phospo-specific antibodies. A possible model for the role of MPS1 could be as follows. In response to unattached kinetochores MPS1 is (auto)phosphorylated and binding (and phosphorylation) to MAD1 occurs. This phosphorylation of MPS1 (and MAD1) recruits the MAD1-MAD2 complex to the kinetochore (Zhao and Chen, 2006) and possibly also recruits other mitotic checkpoint proteins. The formation of a MPS1-MAD-MAD2 complex possibly already occurs at the nuclear pore complex (NPC), as MPS1, MAD1, MAD2 and other checkpoint proteins have been shown to localized at the NPC during interphase in both yeast and vertebrates (Campbell et al., 2001; Iouk et al., 2002; Liu et al., 2003; Stukenberg and Macara, 2003). Furthermore it is suggested, so far only in human cells, that MPS1 is required to recruit O-MAD2 to the MAD1-C-MAD2 complex to further activated the checkpoint (Hewitt et al., 2010).

***No MPS1 homologue in C. elegans?*** So far, the role of MPS1 in the mitotic checkpoint seems to be conserved from yeast to human. However, until today no MPS1 homologue in *C. elegans* could be identified. Kitagawa suggests that this is due to a highly diverse sequence of *C. elegans* MPS1 compared to other MPS1 homologues as worms seem to have an accelerated rate of evolution (Gamulin et al., 2000; Kitagawa, 2009). In this case, an interaction with MAD1-MAD2 would still be expected and rather than on primary amino acid sequence the true *C. elegans* MPS1 could be identified by investigating the proteins interacting with the MAD1-MAD2 complex. To identify the function, these genes could be depleted to see whether the mitotic arrest in *C. elegans* is altered. When the identified protein is indeed a functional homologue, introduction of the protein in MPS1 depleted cells from

other organisms should result in a rescue of the checkpoint. It is also possible that MPS1 is indeed not present and not required in *C. elegans*. The accelerated rate of evolution could also result in the alteration of MPS1 targets in such a way that these targets do not need the interaction or phosphorylation by MPS1 anymore. Another important difference between *C. elegans* and other eukaryotes are the holocentric chromosomes of *C. elegans*, in which the chromosomes act as the centromere over the whole length. It is possible that due to this "bigger" centromeres, the mitotic checkpoint can find the kinetochores "easier" and MPS1 is not needed to recruit them. If MPS1 is indeed required to recruit O-MAD2 to the MAD1-C-MAD2 complex to further activated the checkpoint, also this feature should be different in C. elegans. This could also be due to the holocentric chromosomes, or maybe another protein is required to do this.

***Other roles of MPS1.*** As mentioned, MPS1 was discovered as a protein important in spindle pole body duplication in budding yeast (Winey et al., 1991). Studies in human cells, obtained contradictory results on MPS1 function in spindle pole body duplication, possibly due to antibody use. When comparing the results to mice studies it is most likely that in both mice and human MPS1 is important for duplication of centrosomes, (Fisk et al., 2003; Fisk et al., 2004; Liu et al., 2003; Stucke et al., 2002). Interestingly, MPS1 seems not essential for spindle pole duplication in fission yeast and *Drosophila*, suggesting this MPS1 function is not completely conserved. (Fischer et al., 2004; Fisk and Winey, 2004; He et al., 1998). Next to centrosomes duplication, MPS1 has been shown to play a role in spindle assembly and correcting kinetochore microtubule attachment in both yeast and human cells (Jelluma et al., 2008; Maure et al., 2007) A relationship between MPS1 and Aurora B was suggested to be responsible for this role, but so far contradictory results were obtained concerning the role of MPS1 in Aurora B activation and vice versa. (Bourhis et al., 2009; Jelluma et al., 2008; Maciejowski et al., 2010; Maure et al., 2007; Sliedrecht et al., 2010). Interestingly, Maciejowski et al. also demonstrates that MPS1 regulates BUB1 and Shugoshin, providing another link between MPS1 and chromosomal congression (Maciejowski et al., 2010). As this last result is only obtained in human cells so far, it would be interesting to see the same result in other organisms as the BUB-Shugoshin interaction is conserved from yeast to human.

In summary, MPS1 homologues have been found in all eukaryotes studied, except in C. elegans, and all homologues were required for proper mitotic checkpoint signaling. MPS1 is required for the recruitment of MAD1 and MAD2 (and possibly the other mitotic checkpoint proteins) to the kinetochore.

**Rod-ZW10-Zwilch-complex**

Rough Deal (ROD) and Zeste-White 10 (ZW10) were originally identified in *Drosophila* and mutations have been shown to interfere with the correct transmission of chromosomes to daughter cells during mitosis (Karess and Glover, 1989). These proteins are conserved among other multicellular eukaryotes, like *C. elegans*, *Xenopus*, mouse and human (Chan et al., 2000; Starr et al., 1997). In *Drosophila, C. elegans* and vertebrate cells, deleting both genes resulted in chromosome segregation defects, resulting in aneuploidy (Karess and Glover, 1989; Scaerou et al., 2001; Starr et al., 1997). In addition, the two proteins were shown to be required for the mitotic checkpoint as in *Drosophila*, human and *Xenopus*, cells without ROD or ZW10 did not arrest in prometaphase in response to spindle poisons (Basto et al., 2000; Chan et al., 2000; Kops et al., 2005). ROD and ZW10 were shown to form a conserved complex and to depend on each other's kinetochore localization (Chan et al., 2000; Kops et al., 2005; Scaerou et al., 2001; Williams and Goldberg, 1994; Williams et al., 2003). In both *Drosophila* and human a third gene associated with ROD and ZW10 was identified, Zwilch (Kops et al., 2005; Williams et al., 2003), and together they form the RZZ-complex. Zwilch mutations caused the same phenotypes, aneuploidy and a defective spindle checkpoint, as detected for mutations in the other two proteins (Williams et al., 2003). Furthermore, Zwilch kinetochore localization requires ZW10 and ROD and vice versa (Williams et al., 2003). ZW10 interacting protein 1 (Zwint-1) is a "receptor" recruiting the RZZ-complex to the kinetochore in human and *Xenopus*, but so far no homologue was found in *Drosophila* and *C. elegans* (and not in yeast) (Figure 12) (Gassmann et al., 2008; Kops et al., 2005; Starr et al., 2000; Wang et al., 2004). Whether the RZZ proteins already form a complex before kinetochore localization is not known, as in humans ZW10 and ROD could be separately identified at kinetochores in early prometaphase (Basto et al., 2004; Scaerou et al., 2001; Williams et al., 1992). Upon attachment of the microtubules to the kinetochore both ROD and ZW10 "migrate" along the attached microtubule to the poles and transition to anaphase results in a loss of the RZZ attached to the microtubule, while some RZZ remains present at the kinetochores of the migrating chromosomes (Figure 12) (Basto et al., 2004; Scaerou et al., 1999; Williams et al., 1992).

***Recruitment of MAD1-MAD2.*** A conserved role for the RZZ-complex in the recruitment of MAD1-MAD2 to the unattached kinetochores has been demonstrated in Drosophila, *Xenopus* and human (Figure 12) (Buffin et al., 2005; Kops et al., 2005). *Drosophila* ROD and MAD2 were shown to be recruited simultaneously to the kinetochore, and after kinetochore attachment they move simultaneously along the microtubules to the spindle poles (Buffin et al., 2005). MAD2 disappears from the kinetochores as soon as the kinetochore is attached, while the RZZ-complex remains localized until or even during anaphase (Campbell et al., 2001; Chan et al., 2000; Gillett et al., 2004; Scaerou et al., 2001). This suggests that the RZZ-complex is not the only factor regulating MAD1-MAD2 localization, which is consistent with the observations that BUB1 or MPS1 kinase activity are also required for this (Abrieu et al., 2001; Johnson et al., 2004; Meraldi et al., 2004). The mechanisms by with the RZZ-complex regulates MAD2 recruitment is not clear, as no interaction between MAD1-MAD2 and RZZ was detected so far (Kops et al., 2005; Starr et al., 2000; Williams et al., 2003). Whether there is a third protein important for this interaction, or that the kinetochores basis is required needs to be elucidated (Figure 12 - MAD1-MAD2 receptor) (Karess, 2005). An in vitro study, with or without kinetochores components could give an answer to the latter hypothesis. Furthermore, it would be interesting to check involvement of the RZZ complex in MAD1-MAD2 localization in for example *C. elegans*, to investigate the conservation of this mechanism.

***Other functions of the RZZ complex.*** Next to the recruitment of MAD1-MAD2, the RZZ-complex is also required for the recruitment of dynein-dynactin, a complex involved in numerous aspects of intracellular motility. Disruption of dynactin (vertebrate) or mutations in dynein (flies) result in an overactive mitotic checkpoint, while mutation in the RZZ-complex result in no checkpoint activation at all (Starr et al., 1998; Williams et al., 2003). This suggests that the interaction between these two is not likely to be required for checkpoint activation but possibly for checkpoint inactivation (see below) (Howell et al., 2001; Wojcik et al., 2001). A protein identified in *Drosophila* because of its role in checkpoint silencing was Spindly (Griffis et al., 2007). In *Drosophila*, Spindly recruitment to the kinetochores depends on the RZZ-complex. When localized to the kinetochores Spindly, together with dynactin, is needed to recruit dynein to the spindle poles. In response, dynein recruits Spindly, MAD2 and the RZZ complex to the poles (Figure 12) (Griffis et al., 2007). Similar roles in mitotic checkpoint activation for the human Spindly as for the *Drosophila* Spindly were demonstrated, suggesting a conserved dynein recruitment mechanism

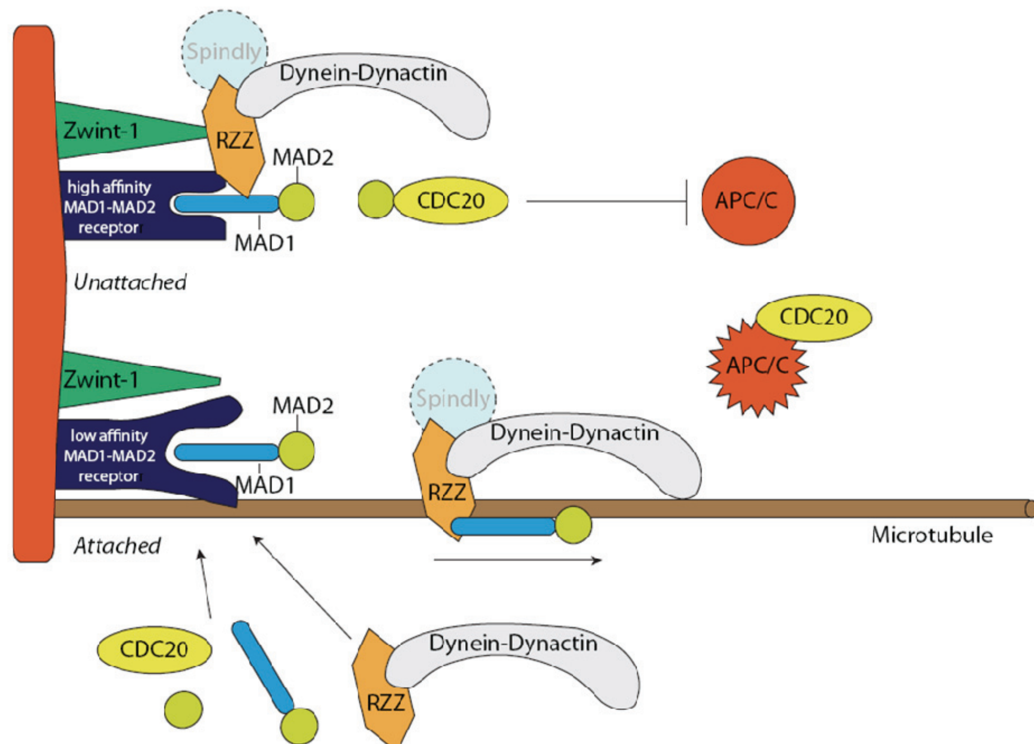


FIG. 12. A model for the role of the RZZ complex at the kinetochore. Unattached: RZZ-complex binds to its receptor Zwint-1 and recruits Dynein-Dynactin. The RZZ-complex recruits Spindly (the role of spindly is not established very well yet) and stabilizes the localization of MAD1-MAD2 by (direct or indirect) binding. Bound MAD1-MAD2 promotes CDC20-MAD2 generation, resulting in APC/C inhibition. Attached: The MAD1-MAD2 receptor loses affinity and MAD1-MAD2, RZZ-complex (and Spindly) are recruited away from the kinetochore by Dynein-Dynactin. No CDC20-MAD2 is generated anymore and the APC/C is activated. New RZZ-complex is continuously distributed to the attached kinetochore to remove any new MAD1-MAD2. When the microtubule destabilizes, new MAD1-MAD2 can immediately assure new APC/C inhibition. Adapted from Karess, 2005.

(Barisic et al., 2010; Griffis et al., 2007). However, in *C. elegans* Spindly (SPLD-1) was shown to be responsible for the recruitment of MAD2 (by being a kinetochore receptor for MAD1) and for the activation of the checkpoint (Gassmann et al., 2008). This results is in contrast with the results obtained in *Drosophila* and human, suggesting dynein functions may actually have diverged during evolution (Barisic et al., 2010).

***No RZZ-complex in yeast*.** In the yeast genome no proteins were found with detectable homology to ZW10, ROD and Zwilch (Williams et al., 2003). Also no Spindly or Zwint-1 was detected. Dynein is excluded from budding yeast nuclei and no NEB occurs during yeast mitosis (Cottingham et al., 1999; Yeh et al., 1995). This suggest that dynein is not required for proper mitosis in yeast, which is in favor with the fact that no RZZ-complex is present and suggests other motor proteins are used in yeast. However, the most important role of the RZZ-complex the mitotic checkpoint is stable MAD1-MAD2 localization to the kinetochore, which is also important in yeast. Possibly another anchor is present in yeast to recruit and stabilize MAD1-MAD2 kinetochore localization or the suggested (unidentified) "MAD1-MAD2 receptor" is capable of stably binding MAD1 and MAD2 without the help of the RZZ-complex.

To be brief, the RZZ-complex is required for proper checkpoint activation in higher eukaryotes, by mediating the recruitment of the MAD1-MAD2 complex. The mechanisms by with the RZZ-complex regulates this recruitment is not clear, as no interaction between MAD1-MAD2 and RZZ was detected so far.

## Silencing the checkpoint

The inactivation of the checkpoint is linked to the correct attachment of the microtubules. Although it is not the main focus of this review, I would like to mention the silencing factors and their conservation shortly.

***Dynein motility: Stripping the kinetochore.*** The recruitment of MAD2, the RZZ complex, dynein/dynactin, BUBR1 and CENP-E to the spindle poles after microtubule attachment seemed to be an important, partially conserved process in the inactivation of the mitotic checkpoint (Figure 12) (Basto et al., 2004; Hoffman et al., 2001; Howell et al., 2001; Wojcik et al., 2001). Interestingly, as discussed above, no RZZ complex, CENP-E or nuclear dynein is present in budding yeast. This suggest that yeast needs another silencing mechanism or another microtubule motor (possibly Kar3) to generate checkpoint inactivation. (Musacchio and Salmon, 2007).

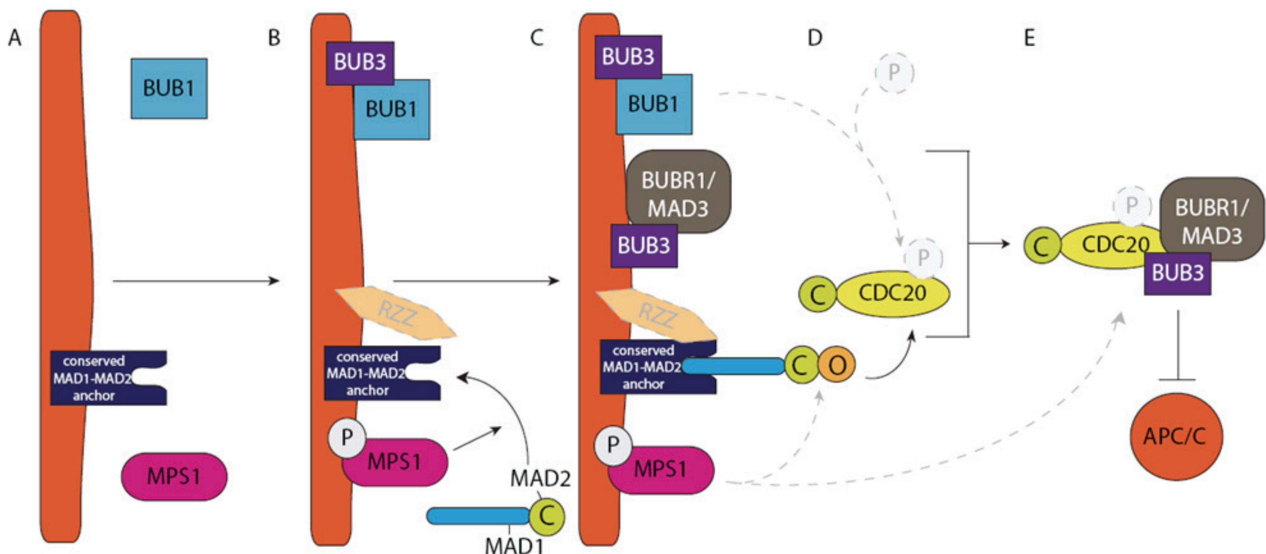


FIG. 13. The core mitotic checkpoint. (A) No mitotic checkpoint proteins are localized to the kinetochore, excepts for a possible MAD1-MAD2 anchor. (B) When MPS1 gets (auto)phosphorylated and BUB3 binds to BUB1 the proteins localize to the unattached kinetochore. Also the RZZ-complex (in higher eukaryotes) will localize to the kinetochore. (C) BUB1 and MPS1 recruit BUBR1/MAD3, MAD1 and MAD2 to the kinetochore. The RZZ-complex stabilizes MAD1-MAD2 kinetochore binding to the anchor. (D) C-MAD2 binds O-MAD2, and O-MAD2 interacts with CDC20 and becomes C-MAD2. MPS1 seems to be required for the recruitment of O-MAD2. In higher eukaryotes, BUB1 phosphorylates CDC20 to enhance APC/C inhibition. (E) The interaction between MAD2 and CDC20 results in the interaction of BUBR1/MAD3 with CDC20 and the formation of the mitotic checkpoint complex. MPS1 seems to be required for the formation of the MCC. The mitotic checkpoint complex inhibits APC/C activity. Grey RZZ-complex, arrows and phosphorylation are not completely conserved or not yet well established.

***Inactivation by p31 comet.*** P31comet is found in human, mice and *Xenopus*, but no obvious homologues were identified in lower eukaryotes like yeast, *C. elegans* and *Drosophila* (Habu et al., 2002; Musacchio and Salmon, 2007; Xia et al., 2004). P31comet interacts with C-MAD2, blocking the ability of O-MAD2 to interact with C-MAD2 and maintain the checkpoint. (Xia et al., 2004; Yang et al., 2007). As p31comet is not needed during normal cell division it possibly is an extra mechanism to control the activation/inactivation of the mitotic checkpoint due to spindle damage in higher eukaryotes. (Xia et al., 2004).

***Ubiquitination of CDC20 and fast checkpoint inactivation.*** The binding of BUBR1/MAD3 to CDC20 has been shown to result in APC/C mediated CDC20 ubiquitination (King et al., 2007b; Pan and Chen, 2004; Reddy et al., 2007). Diaz-martinez et al. proposed that both ubiquitination and deubiquitination of CDC20 are needed to generate a CDC20 balance during spindle checkpoint APC/C inhibition. Upon microtubule attachment, the balance flips to CDC20 ubiquitination resulting in a fast inactivation of the checkpoint (Diaz-Martinez and Yu, 2007). The observation that also p31comet is capable to induce CDC20 ubiquitination is consistent with this model (Reddy et al., 2007).

***Silencing by PP1: a phosphatase opposing Aurora B activity***. Protein Phosphatase 1 (PP1) is a phosphates opposing Aurora B kinase activity, and it was proposed (and proven in budding yeast and fission yeast) that dephosphorylation of Aurora B substrates by PP1 leads to mitotic exit. (Fuller and Stukenberg, 2009; Pinsky et al., 2009; Vanoosthuyse and Hardwick, 2009).

The silencing by PP1 possibly results in stronger silencing by CDC20 ubiquitination, p31 comet and dynein mediated kinetochore stripping. It seems that higher eukaryotes need all four mechanisms, lower eukaryotes need three and yeast only need two (or three if Kar3 is indeed a dynein homologue) mechanism. In this hypothesis, the complexity of the mitotic division, mitotic checkpoint activation is consistent with the complexity of mitotic checkpoint silencing in these species.

## The core mitotic checkpoint proteins

So far I discussed the conservation of mitotic checkpoint proteins and their function (see Supplementary Table 1 for summary), and a core mitotic checkpoint can be proposed (Figure 13). The main inhibitors of the APC/C are MAD2 and BUBR1/MAD3 and the interaction with CDC20 is responsible for this inhibition. It is most likely that in all organisms studied, MAD2 and BUBR1/MAD3 form a complex together with BUB3 and CDC20 (Figure 13E). Efficient formation of this mitotic checkpoint complex requires kinetochore localization, which is accomplished by MAD1 (for MAD2) and BUB3 (for BUBR1) (Figure 13C). Next to kinetochore localization, the interaction between MAD1 and MAD2 results in a MAD2 conformational change, which is required for the interaction with CDC20. The kinetochore localization of MAD1 and BUB3 on their turn is regulated by phosphorylated-MPS1 (for MAD1) and a BUB1-BUB3 interaction (Figure 13B). How MPS1 and BUB1 exactly control this MAD1 and BUB3 kinetochore localization remains to be elucidated.

## Conclusion and future directions

The mitotic checkpoint complex is a well conserved mechanism from yeast to human. Although it seems that every organism has some specific small differences (due to different mitotic timing, different amount of chromosomes, different amounts of cytoplasm etc.) and that higher eukaryotes need extra mechanisms to regulate the checkpoint, the core idea remains the same. Comparing the results obtained in all organisms gives new insides in what features of the mitotic checkpoint are not yet well established and need more research. Whether the MCC in higher eukaryotes is indeed a complex of MAD2-CDC20-BUBR1-BUB3 is an important factor that needs to be established. Although it seems most likely that one MCC is formed, proper results are needed to prove this.

Furthermore, it is of high importance to know the exact function of all the proteins known to contribute to MCC formation. BUB1 for example is required for the kinetochore localization of other checkpoint proteins. How this localization is regulated by BUB1 and if this is indeed its main function needs to be studied. The same is true for MPS1, that is needed for MAD1 recruitment to the kinetochore. Whether MPS1 regulates this recruitment by direct binding or by some other event is not known. Furthermore some other functions of MPS1, like the recruitment of O-MAD2 and its requirement for MCC formation were only detected in human cells so far and it would be interesting to see whether this feature is also present in other organisms. Although the role of MAD1 seems to be clear it is not known what keeps MAD1 at the kinetochore. The presence of a conserved MAD1-MAD2 receptor (Figure 13) could be an explanation, but the existence of such a receptor also needs to be established.

Interestingly higher eukaryotes do have extra control mechanisms, like the kinase domain of BUBR1, the RZZ-complex and phosphorylation and binding of BUB1 to CDC20. The exact reason for the need of these extra mechanisms in higher eukaryotes is not known. An explanation could be the fact that a budding yeast or fission yeast kinetochore only has to bind one or three microtubule respectively, while a mammalian kinetochores needs to bind

a bundle with 20-30 microtubules (Ding et al., 1993; Rieder, 1982; Winey et al., 1995). Another explanation could be that the extra mechanisms are needed to amplify the signals to the cytoplasm in higher eukaryotes, as they have an open mitosis instead of a closed mitosis (like yeast).

Getting a clear overview of the mitotic checkpoints in different organisms is important for the further research of the involvement of the mitotic checkpoint in cancer, as it is very important that feature therapies, targeting the MCC, will act the same way in a model organism as it does in a human being.

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| Name and function | Hs | Xl | Dm | Ce | Sc | Sp | References |
|---|---|---|---|---|---|---|---|
| **MAD2** | √ | √ | √ | √ | √ | √ | (Musacchio and Salmon, 2007) |
| • Essential for mitotic checkpoint signaling | √ | √ | √ | √ | √ | √ | (Buffin et al., 2007; Chen et al., 1996; Dobles et al., 2000; Gorbsky et al., 1998; Hoyt et al., 1991; Kitagawa, 2009; Li and Murray, 1991; Li and Benezra, 1996) |
| • Required for normal cell cycle progression | √ | √ | X | X | X | X | (Chen et al., 1996; Dobles et al., 2000; Gorbsky et al., 1998) |
| • Interaction with and inhibition of CDC20 | √ | √ | √ | √ | √ | √ | (Fang et al., 1998; Hwang et al., 1998; Kim et al., 1998; Kitagawa et al., 2002; Li et al., 2010; Li et al., 1997; Sczaniecka et al., 2008) |
| • Required for CDC20-BUBR1 interaction | √ | √ | X | ? | √ | X | (Chen et al., 1999; Fang et al., 1998; Hwang et al., 1998; Li et al., 2010; Millband and Hardwick, 2002) |
| • Interaction with MAD1 | √ | √ | √ | √ | √ | √ | (Campbell et al., 2001; Chen et al., 1999; Chen et al., 1998; Ikui et al., 2002; Kitagawa and Rose, 1999; Li et al., 2010) |
| • Existence of an open and closed conformation | √ | √ | √ | ? | √ | ? | (De Antoni et al., 2005; Li et al., 2010; Luo et al., 2000; Luo et al., 2004; Nezi et al., 2006; Sironi et al., 2002; Yang et al., 2008) |
| | | | | | | | |
| **MAD1** | √ | √ | √ | √ | √ | √ | (Musacchio and Salmon, 2007) |
| • Essential for mitotic checkpoint signaling | √ | √ | √ | √ | √ | √ | (Chen et al., 1998; Encalada et al., 2005; Hardwick and Murray, 1995; Jin et al., 1998; Kitagawa and Rose, 1999; Luo et al., 2002) |
| • Required for MAD2 kinetochore localization | √ | √ | √ | ? | √ | √ | (Chen et al., 1999; Chen et al., 1998; Chung and Chen, 2002; De Antoni et al., 2005; Howell et al., 2004; Li et al., 2010; Luo et al., 2002) |
| | | | | | | | |
| **BUBR1/MAD3** | BUBR1 | BUBR1 | BUBR1 | MAD3 | MAD3 | MAD3 | (Musacchio and Salmon, 2007) |
| • Essential for mitotic checkpoint signaling | √ | √ | √ | √ | √ | √ | (Chan et al., 1999; Chen, 2004; Hoyt et al., 1991; Li and Murray, 1991; Moore et al., 2005; Nystul et al., 2003; Rahmani et al., 2009) |
| • Required for normal cell cycle progression | √ | √ | X | X | X | ? | (Buffin et al., 2007; Meraldi et al., 2004; Stein et al., 2007; Warren et al., 2002) |
| • Inhibition CDC20 (via KEN-box 1) | √ | √ | √ | ? | √ | √ | (Burton and Solomon, 2007; Hardwick et al., 2000; King et al., 2007b; Sczaniecka et al., 2008; Tang et al., 2001) |
| • Required for CDC20-MAD2 interaction | √ | √ | X | ? | X | √ | (Chen, 2002; Fang, 2002; Hwang et al., 1998; Li et al., 2010; Millband and Hardwick, 2002) |
| • Interaction with BUB3 required for kinetochore localization | √ | √ | ? | ? | √ | √ | (Chen, 2002; Hardwick et al., 2000; Millband and Hardwick, 2002) |
| • Kinase activity is required for proper mitotic checkpoint signaling | CD | CD | √ | n/a | n/a | n/a | (Chen, 2002; Huang et al., 2008; Kops et al., 2004; Mao et al., 2003; Rahmani et al., 2009; Tang et al., 2001) |
|     • via long-term maintanance | √ | √ | √ | n/a | n/a | n/a | (Chen, 2002; Huang et al., 2008; Kops et al., 2004; Mao et al., 2003; Rahmani et al., 2009; Tang et al., 2001) |
| • Interaction with CENP-E is essential for checkpoint signaling | X | √ | X | n/a | n/a | n/a | (Abrieu et al., 2000; Maia et al., 2007; McEwen et al., 2001; Tanudji et al., 2004; Yao et al., 2000) |

Supplementary Table 1. Summary of the mitotic checkpoint proteins and their functions in different model organisms. Hs, *Homo sapiens;* xl, *X. laevis*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans;* Sc, *Schizosaccharomyces cerevisiae*; sp, *Schizosaccharomyces pomb;* √, yes/present; X, no/absent; ?, not studied; CD, conflicting data; n/a, not applicable; some, partial requirement.

| Name and function | Hs | Xl | Dm | Ce | Sc | Sp | References |
|---|---|---|---|---|---|---|---|
| **BUB3** | √ | √ | √ | √ | √ | √ | (Campbell and Hardwick, 2003; Larsen and Harrison, 2004) |
| • Essential for mitotic checkpoint signaling | √ | √ | ? | √ | √ | X | (Kitagawa, 2009; Klebig et al., 2009; Logarinho et al., 2008; Roberts et al., 1994; Windecker et al., 2009; Yao et al., 2000) |
| | | | | | | | |
| Formation of a MAD2-CDC20-BUBR1/MAD3-BUB3 complex | CD | X | ? | ? | √ | √ | (Fang, 2002; Fraschini et al., 2001; Millband and Hardwick, 2002; Sudakin et al., 2001; Tang et al., 2001) |
| | | | | | | | |
| **BUB1** | √ | √ | √ | √ | √ | √ | (Bernard et al., 1998; Chen, 2004; Encalada et al., 2005; Farr and Hoyt, 1998; Logarinho et al., 2004; Meraldi and Sorger, 2005; Perera et al., 2007; Tang et al., 2004a; Warren et al., 2002) |
| • Essential for mitotic checkpoint signaling | √ | √ | √ | √ | √ | √ | (Bernard et al., 1998; Chen, 2004; Encalada et al., 2005; Farr and Hoyt, 1998; Logarinho et al., 2004; Meraldi and Sorger, 2005; Perera et al., 2007; Tang et al., 2004a; Warren et al., 2002) |
| • Required for MAD1, MAD2 and BUBR1-MAD3 localization | √ | √ | ? | ? | √ | ? | (Gillett et al., 2004; Johnson et al., 2004; Meraldi et al., 2004; Sharp-Baker and Chen, 2001) |
| • Interaction with BUB3 required for kinetochore localization | √ | X | ? | √ | √ | X | (Campbell and Hardwick, 2003; Kitagawa, 2009; Klebig et al., 2009; Roberts et al., 1994; Warren et al., 2002; Windecker et al., 2009) |
| • Kinase activity is required for proper mitotic checkpoint signaling | some | some | ? | ? | X | some | (Chen, 2004; Fernius and Hardwick, 2007; Klebig et al., 2009; Roberts et al., 1994; Warren et al., 2002; Yamaguchi et al., 2003) |
| • Interaction with and phosphorylation of CDC20 | √ | ? | ? | ? | ? | ? | (Kang et al., 2008; Tang et al., 2004a; Vanoosthuyse and Hardwick, 2005) |
| • Conserved motif I present | √ | √ | √ | ? | √ | √ | (Klebig et al., 2009; Warren et al., 2002) |
| • Motif required for checkpoint signaling | √ | ? | ? | ? | √ | ? | (Klebig et al., 2009; Warren et al., 2002) |
| | | | | | | | |
| **MPS1** | √ | √ | √ | X | √ | √ | (Abrieu et al., 2001; Fischer et al., 2004; He et al., 1998; Liu et al., 2003; Weiss and Winey, 1996) |
| • Essential for mitotic checkpoint signaling | √ | √ | √ | n/a | √ | √ | (Abrieu et al., 2001; Fischer et al., 2004; He et al., 1998; Liu et al., 2003; Weiss and Winey, 1996) |
| • Required for checkpoint protein kinetochore localization (at least MAD1 and MAD2) | √ | √ | √ | n/a | √ | ? | (Abrieu et al., 2001; Hardwick et al., 1996; Lince-Faria et al., 2009; Maciejowski et al., 2010; Vigneron et al., 2004; Wong and Fang, 2007) |
| • Required for MAD2-CDC20 interaction | √ | ? | ? | n/a | √ | ? | (Hwang et al., 1998; Sliedrecht et al., 2010) |
| • Phosphorylation of MAD1 | CD | ? | ? | n/a | √ | ? | (Campbell et al., 2001; Hardwick and Murray, 1995; Hardwick et al., 1996) |
| | | | | | | | |
| **RZZ-complex** | √ | √ | √ | √ | X | X | (Chan et al., 2000; Karess and Glover, 1989; Okamura et al., 2001; Starr et al., 1997) |
| • Essential for mitotic checkpoint signaling | √ | √ | √ | ? | n/a | n/a | (Basto et al., 2000; Chan et al., 2000; Kops et al., 2005; Scaerou et al., 2001; Starr et al., 1997) |
| • Required for recruitment of MAD1-MAD2 to the kinetochore | √ | √ | √ | ? | n/a | n/a | (Buffin et al., 2005; Kops et al., 2005) |

Supplementary Table 1 (continue). Summary of the mitotic checkpoint proteins and their functions in different model organisms. Hs, *Homo sapiens;* xl, *X. laevis*; Dm, *Drosophila melanogaster*, Ce, *Caenorhabditis elegans;* Sc, *Schizosaccharomyces cerevisiae*; sp, *Schizosaccharomyces pomb;* √, yes/present; X, no/absent; ?, not studied; CD, conflicting data; n/a, not applicable; some, partial requirement.