Inhibiting the inhibitor: how the Spindle Assembly Checkpoint gets silenced

Introduction

For all living creatures on earth it is essential that cells multiply themselves. In order to multiply, cells go through a cell cycle which generally consists of four phases. In the G1 phase, cells grow and prepare to go into the second phase, the so-called S-Phase. In this stage, DNA synthesis takes place to duplicate the chromosomes. During G2 phase, a second period of growth occurs to prepare for the M-phase. In this period, the chromosomes are being segregated by a process called mitosis and finally, cytokinesis divides the cell into two equal daughter cells. The major molecules that drive the cell cycle are Cyclins and Cyclin-Dependent Kinases (CDKs). Cyclin protein levels fluctuate throughout the cell cycle and are required to activate the Cyclin-Dependent Kinases. Different Cyclin/CDK combinations induce different phase transitions.

Entry into mitosis is induced by Cyclin B dependent activation of CDK1[22]. During mitosis, chromosomes attach to microtubules via the kinetochore, a protein complex at the centromere. Each chromosome needs to establish a bipolar attachment, meaning that each copy connects to microtubules from opposing poles. An essential role in this is played by cohesin, which is a ring enclosing sister chromatids to prevent their premature separation. Once all chromosomes are attached during prometaphase, the chromosomes move to the middle of the cell on a so-called metaphase plate. During anaphase, the actual separation of sister chromatids takes place by moving sister chromatids to opposite sides of the cell. The metaphase-to-anaphase transition is triggered by the degradation of two important proteins, Securin and cyclin B[25, 26]. Securin needs to be degraded to release Separase, resulting in cleavage of cohesions and subsequent chromosome separation. Cyclin B breakdown causes CDK1 inactivation, and thus mitotic exit.

The degradation of Securin and Cyclin B is primed by the APC/C (Anaphase Promoting Complex/Cyclosome), an E3 ubiquitin ligase that targets Securin and Cyclin B for destruction by poly-ubiquitination (for review see [27, 28]). The APC/C is a large protein complex of approximately 12 subunits. The E3 ligase can be seen as a platform responsible for the capture and presentation of the substrates to E2 ubiquitin conjugating enzymes, which are responsible for the mono- and poly ubiquitination of substrates. The two best known E2 conjugating enzymes are UbcH5 and UbcH10. The recognition of the APC/C substrates is based on D- and KEN destruction boxes and is most likely mediated by the two APC/C co-activators, CDC20 and CDH1, that are known to be essential for APC/C-mediated destruction of APC/C targets (for review see [29, 30]). Both co-activators have a C-terminal WD40 domain that recognizes the destruction boxes. The binding of these co-activators to the APC/C is transient and tightly regulated. CDC20 is present during mitosis and is necessary for APC/C dependent degradation of Cyclin B and Securin, CDH1 is required for the completion of mitosis and is activated when CDK1 activity decreases. Although, it is generally thought that the co-activators contribute to substrate recruitment, they might also induce a conformational change in the APC/C complex that is required for its activity but many details are lacking to fully explain how the APC/C and its co-activators function together.

To equally divide the genetic material over to daughter cells, anaphase should be only initiated when all sister chromatids are attached to the microtubule spindle. Therefore, the APC/C is inhibited until all kinetochores are attached. The cell has evolved a surveillance mechanism, called the mitotic checkpoint or Spindle Assembly Checkpoint (SAC), that senses unattached kinetochores and translates this into inhibition of APC/C activity. Proper timing of the APC/C activation is very important to prevent chromosome mis-segregation since premature anaphase onset could lead to an unequal division of chromosomes resulting in two aneuploid daughter cells. Aneuploidy is a major cause of birth defects and chromosomal disorders like down syndrome[31] and is also thought to contribute to tumor formation, as most human tumors contain cells with abnormal amounts of chromosomes[31, 32]. To prevent chromosome mis-segregation, the Spindle Assembly Checkpoint causes a mitotic arrest in cells containing unattached kinetochores through inhibition of CDC20-dependent APC/C activation. The robustness of the checkpoint is illustrated by the fact that the occurrence of even one single unattached kinetochore is sufficient to induce a mitotic arrest[33]. Intriguingly, the checkpoint is almost immediately inactivated after the attachment of the last kinetochore[25]. In this review, the mechanisms for this checkpoint silencing are being discussed.

Understanding the Spindle Assembly Checkpoint

In order to understand the mechanisms of checkpoint silencing, it is important to first look at how the spindle checkpoint induces a mitotic arrest. Therefore, the mechanisms of checkpoint activation and maintenance are briefly discussed in this section. For a broader review on this subject see [34].

The proteins important for the Spindle Assembly Checkpoint, MPS1, BUB1, BUB3, MAD1, MAD2 and MAD3 (also known as BUBR1 in higher eukaryotes), were originally identified in yeast but are highly conserved throughout evolution and are also required for spindle checkpoint function in higher eukaryotes[35-37]. Of these proteins, MAD2, BUB3, BUBR1 and MPS1 are highly dynamic at the unattached kinetochore whereas MAD1 and BUB1 are relatively stable[2]. The fact that all checkpoint proteins interact with the unattached kinetochore strongly suggests that the kinetochore is the major catalytic platform of checkpoint activation where inhibitors of the APC/C are produced. It is thought that the inhibitor modified by the unattached kinetochore is a complex of MAD2, BUBR1 and BUB3 that bind CDC20 and associates tightly with the APC/C in almost equimolar levels[38-40]. The inhibitor complex, called the Mitotic Checkpoint Complex, is already present in low levels during interphase, before the kinetochores are assembled[38, 41, 42], suggesting that the initial levels of mitotic checkpoint complexes is sufficient to inhibit the APC/C until the kinetochore is matured and able to produce high levels of inhibitory complexes[43]. Although it is known that MAD2 is required for the formation of APC/C inhibitor complexes, recent studies have challenged whether MAD2 is also part of the MCC[44, 45]. In these studies it has been suggested that MAD2 binding to CDC20 is required for the formation of a complex consisting of BUBR1, BUB3 and CDC20, since MAD2 promotes the binding of BUBR1 to CDC20[46-48], rather than being part of the complex itself (**FIG. 1A**)[44, 45]. However, Herzog *et al.*[17] recently showed by structural analysis that MAD2, in a stoichiometric complex with BUBR1, BUB3 and CDC20, binds and inhibits the APC/C.

Although the exact composition of the APC/C inhibitory complex is still unclear, it is very likely that binding of the inhibitor complex to the Anaphase Promoting Complex is the major APC/C inhibitory mechanism (**FIG. 1B**). Because of the presence of destruction boxes, it is generally thought that BUBR1 functions as a pseudo-substrate inhibitor that binds the APC/C and prevents binding of APC/C

substrates[40, 46, 49-53]. Structural analysis revealed that the mitotic checkpoint complex associates with the CDC20 binding site of the APC/C and causes repositioning of CDC20, which prevents APC/C activation, as well as conformational changes in APC/C itself, that converts the otherwise flexible APC/C into a "closed" state[40]. Others stated that binding of BUBR1-CDC20 causes APC/C dependent degradation of CDC20, which is thought to facilitate the mitotic arrest[44, 52, 54].

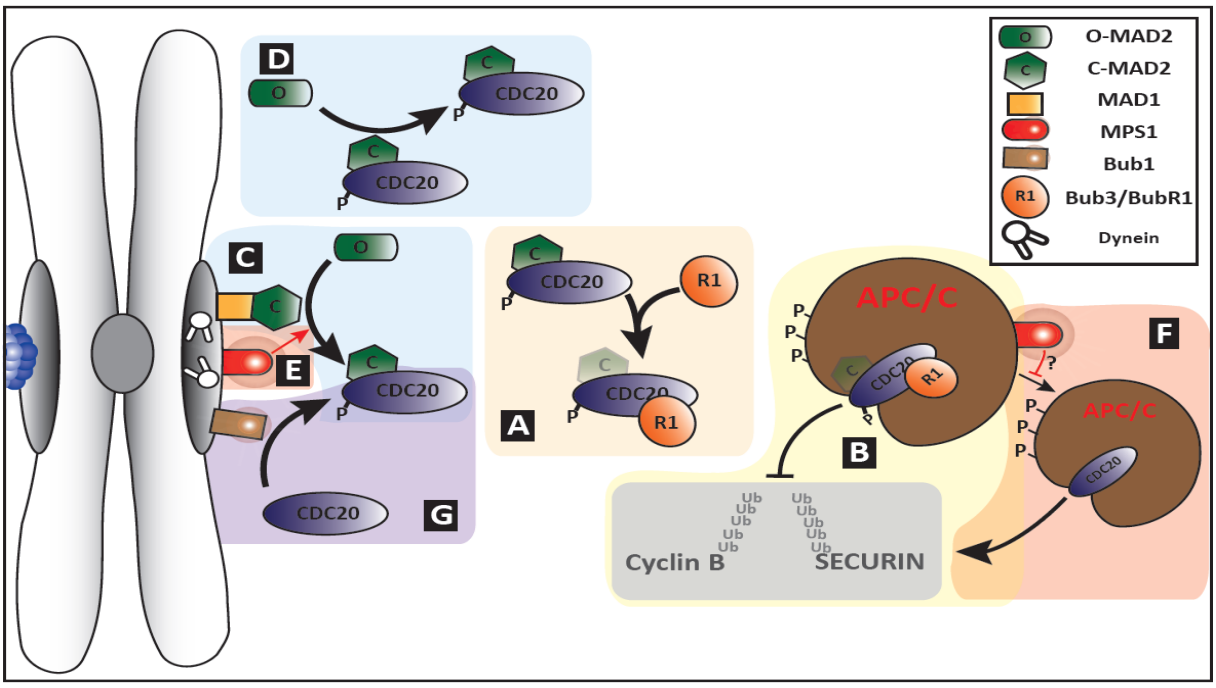

Figure 1 | **Activation of the Spindle Assembly Checkpoint by the unattached kinetochore.** Panel A-G: see text for the description of the figure.

Although it is unclear if MAD2 is part of the APC/C inhibitor complex, it is at least required at the kinetochore to activate the checkpoint. MAD2 binding to the stably bound MAD1 is necessary for its recruitment to the unattached kinetochore[55-57] and for the conformational change that converts MAD2 from inactive O-MAD2 into the active C-MAD2 conformer [57, 58], a change that is important for checkpoint functioning since it accelerates MAD2 binding to CDC20[56, 59]. These facts have lead to two models; an exchange model[58], where binding of O-MAD2 to MAD1 leads to conversion of O-MAD2 into C-MAD2, which in turn binds CDC20, and a template model[60] where MAD1-C-MAD2 serves as a template for the formation of CDC20-C-MAD2. The latter model is supported by the fact that there are two populations of MAD2, one highly dynamic at the kinetochore and the other stably bound to MAD1[60-62], and the fact that the stably bound C-MAD2 is required for the conformational change[60, 63-65]. It is thought that C-MAD2-O-MAD2 dimerization accelerates the rate of MAD2 binding to CDC20, which is suggested to be the rate limiting step (**FIG. 1C**)[11, 66]. It is not yet clear whether C-MAD2-O-MAD2 dimerization leads to conversion of O-MAD2 into I-MAD2, an intermediate form that binds CDC20, or to direct conversion into C-MAD2, via a prion like reaction, which in turn binds to CDC20[67]. In addition to the MAD1-C-MAD2 , it has been suggested that the C-MAD2-CDC20 can also serve as a template for the formation of additional C-MAD2-CDC20 since CDC20 share a similar MAD2 binding motif with MAD1 (**FIG. 1D**)[60, 62, 65, 68]. This hypothesis has been proposed as a mechanism of amplification to expand the number of inhibitory complexes, which might explain why one unattached kinetochore is sufficient to induce checkpoint signaling.

Apart from the proteins that are directly involved in APC/C inhibition, there are also several proteins that are indirectly required for the mitotic checkpoint. For example, rod and zw10, which belong to the RZZ complex, are required for MAD1 and MAD2 recruitment to the unattached kinetochores[69, 70]. Similarly, the kinase MPS1, originally found as a protein involved in centrosome duplication, is dynamically localized at the kinetochore and is, like RZZ, required for the recruitment of at least MAD1-MAD2 to the kinetochore[41, 42, 61, 71-76]. The kinase activity of MPS1 is indispensible for its checkpoint function since a kinase defective mutant has the same phenotype as an MPS1 null mutant[73, 77]. Although the kinetochore localization of MAD1 is only partially dependent on the kinase activity of MPS1, MAD2 recruitment to the MAD1-C-MAD2 core complex is largely dependent on the kinase activity(**FIG. 1E**)[41, 73, 78]. Besides MPS1 function at the unattached kinetochore, it probably also has a function in the cytoplasm, where it might have an effect on the stability of APC/C inhibitor complexes during interphase and mitosis (**FIG. 1F**)[72, 41, 42]. Although it has been suggested that Aurora B might also have a direct role in the checkpoint[79-81], it is more likely that Aurora B induces checkpoint signaling indirectly by creating unattached kinetochores (BOX 1) . Another protein that probably has a function in the checkpoint is BUB1, a kinase that resides at the kinetochore[61]. It has been postulated that BUB1 is important for CDC20 phosphorylation, which is required for the inhibition of the APC/C[82-85]. Phosphorylation of CDC20 might directly inhibit APC/C activation through electrostatic hindrance of the binding of its co-activator but it is more likely that it is required for the formation of CDC20 inhibitor complexes (**FIG. 1G**). However, it still remains unclear what the exact role of CDC20 phosphorylation is in APC/C inhibition.

BOX 1: Error correction

For proper chromosome segregation, both sister chromatids have to be attached to separate spindle poles. However, during search and capture for kinetochores by microtubules, a lot of mis-attachments are made, for example synthelic attachments, where both sister chromatids are attached to the same spindle pole, or merotelic attachments, where one kinetochore is attached to both spindle poles. A mechanism exists to correct these errors. Probably one of the most important proteins for this error correction is Aurora B kinase, a chromosomal passenger protein which promotes biorientation together with other members of the chromosomal passenger complex (CPC), like INCENP, Survivin and Borealin[57, 124, 125]. These proteins function interdependently in localizing the CPC to the inner kinetochore and activate each other[1-8]. Aurora B promotes biorientation in a tension-sensitive manner by promoting the turnover of kinetochore-microtubule attachment[9-12]. In this way, Aurora B indirectly induces the spindle checkpoint by creating unattached kinetochores. In vertebrates, the disassembly of kinetochore-microtubule attachments is probably mediated by Ndc80/Hec1, which is part of the KMN (KNL1, Mis12, Ndc80) network and is involved in microtubule binding. Phosphorylation of Ndc80 by Aurora B reduces the microtubule binding affinity of Ndc80, thereby promoting the creation of unattached kinetochores[13-17]. Upon bi-orientation, tension also has a positive effect on the number and the stability of kinetochore-microtubule attachments[18-20]. Tension probably spatially removes substrates from Aurora B, thereby inhibiting its function of promoting the turnover of kinetochore microtubule attachments[21]. Apart from regulating Ndc80/Hec1, it has also been shown that Aurora B regulates the centromere levels of MCAK, a member of the kinesin-13 family microtubule depolymerizing kinesins[13, 14, 23, 24]. Although MCAK seems to play a role in promoting the turnover and stability of kinetochore-microtubule interactions, it is still unclear what the exact contribution of MCAK is.

What triggers SAC silencing

Once all sister chromatids are bi-orientated, checkpoint silencing is rapidly induced. Inhibition of bi-orientation has been shown to severely delay anaphase onset[86]. There is a long ongoing debate whether attachment or tension triggers checkpoint silencing, which is being discussed in numerous reviews. Because of the interdependency of bi-orientation and checkpoint silencing, it is difficult to discriminate which process is the actual trigger. In two recent elaborate reviews it is argued that attachment triggers SAC silencing[87, 88] while in another it is argued that tension is most important[89]. In this section it is briefly discussed why attachment is probably the inducer of checkpoint inactivation.

Convincing evidence that attachment rather than tension is required for triggering checkpoint silencing comes from laser ablation experiments which showed that ablation of the last unattached kinetochore triggers anaphase onset without creating tension[33]. Further indications that attachment is the actual trigger for inducing SAC silencing comes from an experiment wherein tension is prevented by inducing mitosis without preliminary DNA synthesis. O'Connell *et al.*[90] showed that despite the lack of tension, attachment is sufficient to induce mitotic exit. However, we should note that another independent study from Stern *et al.*[91] showed that absence of replication causes a mitotic delay in budding yeast. However, Stern *et al.*[91] couldn't rule out the role of detachments and spindle abnormalities in the arrested cells.

The first indications that tension might induce checkpoint silencing comes from an experiment where tension is artificially applied in meiotic cells by a micromanipulation needle, resulting in significantly reduced anaphase onset[92]. Furthermore, it has been suggested that tension and not attachment is required for checkpoint silencing because stabilization of microtubules by means of taxol treatment induces a delay in anaphase onset despite bi-orientation[86]. However, anaphase is characterized by the movements of sister chromatids to opposite poles, a process that itself is dependent on microtubule dynamics. Furthermore, studies with other concentration of taxol, where bi-orientation but not tension is permitted, showed that checkpoint silencing is dependent on attachment rather than tension[93]. Likely, tension promotes SAC silencing indirectly by stabilization of attachments and by preventing Aurora B-induced detachments (BOX 1). Probably, kinetochore attachment induce SAC silencing independent of the type of attachments, the lack of tension can re-activate the checkpoint by creating unattached kinetochores when the kinetochores are wrongly attached. This is supported by the fact that synthelic attachment causes SAC silencing when tension-dependent error correction is inhibited[94] and the fact that MAD2 rebinds to the kinetochore upon detachment[19].

Recently, two groups reported another kind of tension, called intrakinetochore tension, which is responsible for kinetochore changes upon microtubule attachments, resulting in an increased distance between inner- and outer-kinetochore proteins[95, 96]. It has been proposed that intrakinetochore tension might regulate localization and/or phosphorylation of checkpoint proteins[87], thereby linking attachment to the Spindle Assembly Checkpoint.

Silencing the Spindle Assembly Checkpoint

Once all kinetochores are attached in a bi-orientated fashion, the spindle checkpoint is rapidly silenced to allow the transition of metaphase to anaphase. In this section, certain proposed mechanisms of checkpoint inactivation will be discussed.

Inhibition of checkpoint proteins

As mentioned before, the production of APC/C inhibitor complexes is required for the activation of the spindle assembly checkpoint and a critical step in this is the formation of MAD2-CDC20 complexes, which might be responsible for cytoplasmic amplification of APC/C inhibitors. It is conceivable that for proper silencing, the production of APC/C inhibitor complexes must be prevented. P31^{COMET}, which is identified in a yeast two-hybrid screen as a MAD2 binding partner, has been put forward as a potential inhibitor of MCC formation. Experiments with P31^{COMET} revealed that depletion significantly delays anaphase[97, 98], while overexpression induces premature chromosome segregation and mitotic exit[1], indicating that P31^{COMET} is involved in the inactivation of the Spindle Assembly Checkpoint, either directly or indirectly. Xia *et al.*[98] showed that P31^{COMET} binds selectively to the closed conformation of MAD2 and enhances the APC/C activity, suggest that P31^{COMET} might prevent binding of C-MAD2 to CDC20 by competitive binding. However, Xia *et al.*[98] showed that CDC20 can form a trimer with C-MAD2 and P31^{COMET}, indicating that P31^{COMET} does not prevent direct C-MAD2 binding to "naked" CDC20 but is more likely to prevent binding of O-MAD2 to C-MAD2, thereby preventing the formation and amplification of CDC20-C-MAD2 complexes. *In vitro* FRAP experiments and competition assays with GST-CDC20-C-MAD2 revealed that P31^{COMET} indeed prevents O-MAD2 recruitment to MAD1-C-MAD2 and CDC20-C-MAD2 complexes, probably by binding with a higher affinity to the bound C-MAD2 than O-MAD2 does[63, 99]. This is supported by the crystal structure of the C-MAD2-P31^{COMET} complex, which revealed that P31^{COMET} blocks MAD2 activation through structural mimicry[100], since P31^{COMET} has been shown to bind at the same place to C-MAD2 as O-MAD2 does, but more closely and with a slightly larger coverage[67, 100]. All these results strongly suggest that both disruption of the initial formation of CDC20-C-MAD2 (**FIG. 2A**), by binding MAD1-C-MAD2, and disrupting amplification of inhibitor complexes (**FIG. 2B**), by binding CDC20-C-MAD2, are required for checkpoint inactivation. Mathematical modeling showed that P31^{COMET} indeed contributes to checkpoint inactivation if P31^{COMET} is activated at the metaphase to anaphase transition, but it is not sufficient for full checkpoint inactivation[101]. A puzzling observation is that P31^{COMET} is already present at the kinetochore in mitotic arrested, nocodazole treated cells[97, 98]. It is not known whether and how P31^{COMET} activity and or binding is regulated during the cell cycle and why P31^{COMET} at the kinetochore does not prevent checkpoint activation. Possibly P31^{COMET} activity is inhibited at the kinetochore via an unknown mechanism and recruited to the unattached kinetochore to prevent cytoplasmic amplification of CDC20-MAD2 complex formation. Although it is evident that there is correlation between MAD2-CDC20 binding and checkpoint inactivation, the Mad1–Mad2–p31^{COMET} interaction does not appear to be regulated during the cell cycle[65].

Another proposed checkpoint silencing mechanism is the CENP-E mediated inhibition of the kinetochore localized BUBR1. From studies with Xenopus egg extracts, it has been proposed that the motor protein CENP-E plays an important role in establishing and maintaining the checkpoint[71, 72, 102-105]. CENP-E stimulates the kinase activity of the kinetochore localized BUBR1 which, in turn, is required for the recruitment of MAD1 and MAD2 to the kinetochore[71, 72, 102, 103]. Upon microtubule

attachment, CENP-E mediated stimulation of BUBR1 kinase activity is ceased[104], resulting in decreased activation of the BUBR1 kinase activity and a subsequent decrease in MAD2 recruitment. In this way checkpoint silencing is linked to microtubule attachments. However, studies in other organisms have questioned whether BUBR1 kinase activity is required for the localization of MAD1 and MAD2. Yeast is capable of recruiting MAD1 and MAD2 whereas the Yeast homolog of BUBR1, MAD3, lacks the kinase domain. In addition, several elegant experiments have shown that BUBR1 kinase activity is dispensable for Spindle Assembly Checkpoint signaling in human, fly and mouse cells[45, 47, 48, 53, 106, 107]. Furthermore, Maia *et al.*[108] and Tanudji *et al.*[109] showed that depletion of CENP-E delays anaphase onset, indicating that CENP-E is not required for the induction of a mitotic arrest. It is therefore likely that the kinetochore localized BUBR1 and its kinase activity is functioning in another process than the mitotic checkpoint. It has been shown that BUBR1 also functions in spindle assembly by regulating microtubule attachments[50, 53, 110-113]. The KEN boxes of BUBR1 that are required for checkpoint signaling are not required for its function in spindle assembly[53], strongly suggesting that BUBR1 has a dual function, on the one hand it functions in checkpoint signaling and on the other hand it functions in supporting spindle assembly. CENP-E mediated activation of BUBR1 kinase activity probably affects checkpoint silencing indirectly by stimulating attachments. This resembles a typical difficulty in studying spindle checkpoint silencing. Because of the intimate link between bi-orientation and checkpoint silencing, it is difficult to discriminate between mechanisms that are required for promoting bi-orientation and mechanisms that are directly involved in SAC silencing.

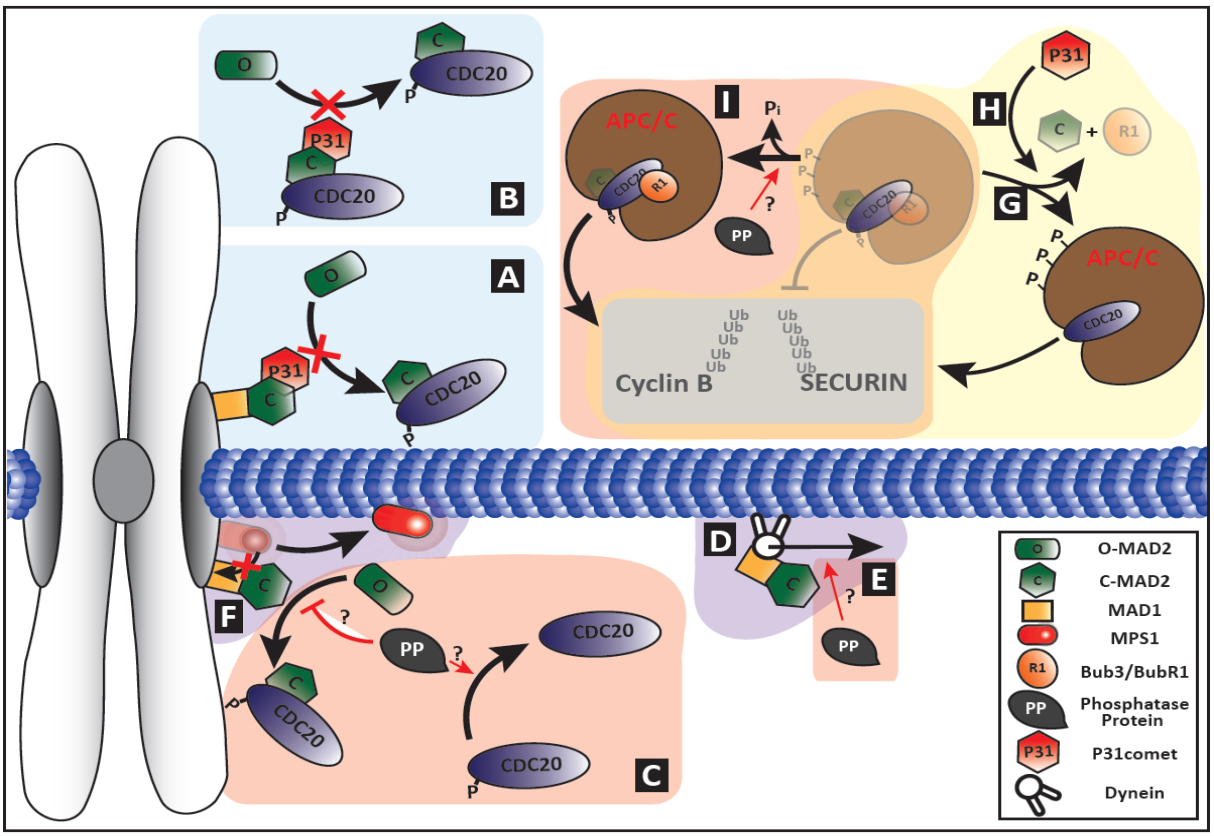

Figure 2 | **Silencing mechanisms of the Spindle Assembly Checkpoint upon attachment.**
Panel A-I: compare with figure 1 and see text for the description of the figure.

The role of phosphorylation in inhibiting MCC formation during checkpoint silencing

As mentioned before, several kinases like MPS1, BUB1 and Aurora B are required directly for checkpoint signaling, by stimulating inhibitor complex, and indirectly, by promoting bi-orientation. The kinase MPS1 and BUB1 are also regulated by kinases themselves[78, 82, 114-121]. As phosphorylation has such a prominent role in spindle checkpoint activation, it is likely that inhibition of phosphorylation, either by inactivating the kinase or by activating a phosphatase, contributes to checkpoint silencing. A protein phosphatase could, for example, counteract the kinase function of BUB1 and MPS1 in the formation of C-MAD2-CDC20 inhibitor complexes (**FIG. 2C**). In Yeast, the homolog of PP1, Gcl7, is identified as such a phosphatase that is required for checkpoint silencing[81, 122], presumably by counteracting Aurora B and MPS1 dependent phosphorylations[122, 123]. The activity of PP1 is probably regulated by its localization since Vanoosthuyse *et al.*[81] showed that for a proper checkpoint silencing function, PP1 requires to be located at the kinetochore. This localization is probably partly dependent on the spindle stabilizing protein Fin1[124], since Akiyoshi *et al.*[124] showed that mislocalization of Fin1 silences the checkpoint in a PP1-dependent manner. Surprisingly, premature spindle checkpoint silencing is not detected in Fin1 depleted cells[124], suggesting that additional factors are involved in activating PP1. Although, PP1 seems to have a role in checkpoint silencing in yeast, there are no studies in higher eukaryotes that identified PP1 as a regulator of checkpoint silencing. It is possible that PP1 mediated checkpoint silencing is specific for yeast but it is also possible that higher eukaryotes have other redundant phosphatases to silence the checkpoint. Either way, the inhibition of the checkpoint by activating dephosphorylation is an attractive mechanism that could contribute to checkpoint silencing. Next to dephosphorylation, it has also been postulated that phosphorylation of MAD2 might contribute to checkpoint silencing as phosphorylation of Serine residues 170, 178 and 195 is said to facilitate checkpoint silencing by modulating its association with MAD1 and CDC20[125]. Analysis of the structure of O-MAD2 and C-MAD2 reported by Mapelli *et al.*[67] revealed that although S187 and S195 are in the C-terminal tail of C-MAD2 (“safety belt”), the residues are not in contact with O-MAD2, suggesting that they are not required for O-MAD2-C-MAD2 dimerization. Serine residue 170 is located at the interface of MAD1 and MAD2 dimerization[62, 67] and is highly conserved between organisms[125], indicating that S170 phosphorylation might indeed affect inhibitor complex formation by modulating the association of MAD2 with MAD1 or CDC20.

Removal of checkpoint protein

Another way of inhibiting formation of inhibitory complexes is by removing certain proteins that are required for checkpoint activation from their habitual location. Since the kinetochore is the basic platform for checkpoint signaling, ”disrupting” the catalytic platform by removing critical proteins could be an efficient mechanism of checkpoint silencing. Hoffman *et al.*[126] showed that the kinetochore levels of certain checkpoint proteins, especially MAD2, drops upon microtubule attachment. Consistent with this, Shah *et al.*[127] showed that, MAD1 and MAD2 are being transported to the spindle poles upon microtubule attachment[127] in a dynein/dynactin-dependent manner[128]. It is likely that MAD1-MAD2 is transported together with Dynein towards the spindle poles (**FIG. 2D**), since kinetochore localized Dynein levels also decrease upon microtubule attachment[126]. It has been suggested that the conformational change of MAD2 might also be induced by MAD1-MAD2 at the spindle pole[127]. However, it is not likely that this conversion could lead to the formation of APC/C inhibitor complexes in the absence of the catalytic platform.

To fully understand the above described stripping process, it is important to know how kinetochore localization of dynein is regulated. Whyte *et al*.[129] identified that dynein phoshorylation is required for its kinetochore localization. They showed that phosphodynein is recruited to the kinetochore by the zw10 subunit of the RZZ complex and that disrupting Dynein dephosphorylation prevents the removal of checkpoint proteins[129], indicating that Dynein dephophorylation might be important for checkpoint silencing. They proposed a model wherein attachment dependent dephosphorylation of dynein causes a shift from binding to zw10 to binding to dynactin, which stimulates dynein for translocation away from kinetochores[129]. The fact that dephosphorylation occurs almost immediately after attachment, suggests that it is dependent on the activity of a phosphatase (**FIG. 2E**). Phosphodynein recruitment to the RZZ complex is probably via Spindly, a protein identified in a RNAi screen in *Drosophila* as being required for SAC silencing[130]. Spindly recruitment is dependent on the RZZ complex and Spindly, in turn, functions in the recruitment of Dynein to the kinetochore, a function that seems to be conserved in all metazoan species[130-133]. Although Spindly-dependent Dynein recruitment is conserved between species, the exact role of Spindly in checkpoint dynein dependent stripping remains unclear. Studies with *Drosophila* showed that Spindly is required for checkpoint silencing by removing MAD2 from the kinetochores[130]. However, studies with *C. elegans* and human Spindly showed that depletion of Spindly does result in checkpoint silencing after a delay in alignment but does not affect MAD2 removal[131-133]. In *C. elegans* and humans it has been shown that Spindly is also required for kinetochore-microtubule interactions[132, 133], explaining the delay in alignment after Spindly depletion. Interestingly, Gassmann *et al.*[133] showed that hSpindly mutants that are affected in dynein recruitment retain at the kinetochore and prevent the kinetochore removal of MAD1, MAD2 and CENP-E upon attachment, suggesting that dynein-dependent removal of Spindly from kinetochores, rather than polewards transport per se, is required for silencing the checkpoint. Because Spindly is required for kinetochore localization of dynein[132, 133] and for kinetochore-microtubule interactions it is likely that Spindly depletion results in MAD2 removal by a kinetochore dynein-independent mechanism following a delayed anaphase onset.

Apart from the removal of MAD2, MPS1 recruitment to unattached kinetochores is also inhibited upon bi-orientation[61], suggesting that removal of MPS1 is also important for checkpoint silencing. Jelluma *et al*.[134] showed that MPS1 removal is indeed required for checkpoint silencing by using a Mis12-Mps1 fusion protein, which prevents the release of MPS1 from the kinetochore. The tethered MPS1 causes a kinase dependent metaphase delay by retaining MAD1-MAD2 at the kinetochore. Possibly, sustained MPS1 activity at the kinetochore prevents checkpoint silencing by stabilizing recruitment of MAD1-MAD2 to the kinetechore, meaning that removal of MPS1 might authorize the Dynein-dependent stripping of MAD1-MAD2 dimers (**FIG. 2F**). It is unclear whether MPS1 release from the kinetochore is dependent on Dynein or whether it is removed via an unknown, kinetochore Dynein-independent mechanism. One such mechanism might be degradation, as shown by Palframan *et al.*[135] in yeast. However, they showed that protein levels of MPS1 fall during anaphase and is dependent on APC/C^{CDC20}- and APC/C^{CDH1} activity, suggesting that degradation of MPS1 is initiated after metaphase-to-anaphase transition when APC/C is already activated. MPS1 degradation is probably required for prevention of SAC re-activation or for mitotic exit itself rather than for checkpoint inactivation.

Complex dissociation

The mechanisms of checkpoint inactivation mentioned before all contribute to reduced formation of APC/C inhibitor complexes. However, unless the inhibitor complexes are very unstable, the already formed inhibitor complexes should be dissassembled (**FIG. 2G**). Based on mathematical modeling, it has been suggested that the release of CDC20 from Mad2 binding is an energy driven process, rather than a spontaneous one[66] and it has been shown that checkpoint silencing indeed requires ATP[136]. The first indications of a complex dissociation mechanism that requires ATP came from Reddy *et al.*[137]. They showed by both *in vitro* and *in vivo* experiments that the spindle assembly checkpoint is inactivated through UbcH10-dependent dissociation of APC/C inhibitor complexes. It has been demonstrated that P31^{COMET}, which activity is correlated with complex dissociation[97, 98], is required to lower the threshold for UbcH10 activity on the APC/C, thereby promoting inhibitor complex dissociation[137] (**FIG. 2H**). Using methylated ubiquitin, two independent studies showed that polyubiquitination rather than mono-ubiquitination is required for the release of MCC from the anaphase promoting complex[136, 137]. Reddy *et al.*[137] and Stegmeier *et al.*[138] proposed that ubiquitination of CDC20 drives disassembly of MAD2-CDC20 complexes and is counteracted by the deubiquitination enzyme USP44, which expression and activity has been shown to be elevated in checkpoint arrested cells and is rapidly degraded upon mitotic exit[138]. These data suggest that checkpoint silencing is triggered by the inactivation of USP44, thereby allowing polyubiquitination of CDC20 and the following release of inhibitor complexes from the APC/C. However, although the CDC20 ubiquitination model is very attractive for APC/C activation, it has been challenged by Nilsson *et al*.[44] who demonstrated that substitution of CDC20 by a non-ubiquitinatable CDC20 does not prevent dissociation of APC/C inhibitor complexes and mitotic exit. Furthermore, this lys-less CDC20 overrides the spindle assembly checkpoint rather than delaying anaphase onset.

There is some debate about the requirement of proteolysis in complex dissociation. Whereas two studies showed, by using the MG132 proteasome inhibitor, that the release of APC/C from its inhibitors is dependent on polyubiquitination rather than degradation[136, 137], another study showed, by using the same proteasome inhibitor, that proteolysis itself is required for complex dissociation[139]. BUBR1, which is also part of the APC/C inhibitor complex, is shown to be degraded during checkpoint inactivation, and might be responsible for checkpoint silencing due to inhibitor complex dissociation[140]. The first indications for this came from siRNA experiments that show that depletion of BUBR1 accelerates mitosis[43]. In an elegant study with ATP analogues, Miniowitz-Shemtov *et al.*[136] showed that, although polyubiquitination and degradation might be required for the release of the inhibitor complexes from APC/C, it is certainly not sufficient to induce checkpoint inactivation. Another energy consuming process is required for the dissociation of inhibitor complexes.

The other energy consuming process is possibly the de-Acetylation of BUBR1. Choi *et al.*[140] showed that BUBR1 is in complex with the lysine acetyltransferase PCAF, which acetylates BUBR1 at K250. Experiments with acetylation mimicking mutants showed that acetylation inhibits BUBR1 ubiquitination and degradation, thereby maintaining a mitotic arrest, whereas acetylation deficient mutants exit mitosis even in presence of nocodazole[140],indicating that BUBR1 degradation is inhibited by acetylation until de-Acetylation allows BUBR1 degradation. However, until now it is unclear what exactly causes the de-acetylation and whether there is a link between de-acetylation and kinotochore attachments. In addition to de-acetylation, active dephosphorylation, by for example PP1, could be another energy consuming process that promotes disassembly of inhibitor

complexes since it has been shown that inhibiting phosphorylation could contribute to complex dissociation[139]. However, Visconti *et al.*[139] showed that inhibiting phosphatases, like PP1, by okadaic acid did not affect complex dissociation, suggesting that although active dephoshorylation by phosphatases is attractive for checkpoint silencing, it is probably not involved in the dissociation of APC/C inhibitor complexes.

Next to inhibitor complex dissociation, it is also possible that the APC/C is released from its inhibition through desensitization of the APC/C for inhibition by the MCC. This is supported by Sudakin *et al.*[38] who have shown that only APC/C purified from mitotic extracts is sensitive for inhibition by the complex, suggesting that not the MCC itself but the APC/C changes during mitosis in a way that it is sensitized for inhibition by the MCC. This sensization could be mediated via Cyclin B/CDK1-dependent phosphorylation of the APC/C[38, 141, 142] and it is therefore quite possible that inhibiting the phosphorylation status of APC/C desensitizes the APC/C, thereby allowing poly-ubiquitination of Cyclin B and Securin even in the presence of the APC/C inhibitor complex (**FIG. 2I**).

Concluding remarks

The mitotic checkpoint is an incredibly complex process and many mechanisms of checkpoint activation and silencing have been identified. However, neither one is sufficient to fully explain the behavior of the Spindle Assembly Checkpoint. Therefore, the question remains whether there is a single major mechanism of checkpoint silencing or whether silencing is regulated by multiple pathways that function together. In yeast, PP1-dependent dephosphorylation is probably the major mechanism of checkpoint silencing since a lot of silencing factors, for example P31^{COMET} and kinetochore localized dynein, have not been identified. Because of the importance of a proper checkpoint silencing, it is likely that multiple pathways evolved in higher eukaryotes that function together to ensure proper silencing. Reviewing these pathways resulted in a model of checkpoint silencing in higher eukaryotes (**FIG. 2**). In this model, attachment of the last kinetochore results in inhibition of inhibitor complex formation and the dissociation of these complexes. Removal of MPS1 probably allows dynein-dependent stripping of checkpoint proteins whose kinetochore localization is critical for the formation of inhibitor complexes. At the same time, attachment licenses P31^{COMET} to prevent the ongoing cytoplasmic amplification by preventing the conformational activation of MAD2. P31^{COMET} has probably a dual role since it also contributes to complex dissociation, which may be mediated by UbcH10-dependent ubiquitination. BUBR1, which is part of the MCC, is one of the proteins that is ubiquitinated by the APC/C and subsequently degraded upon attachment of the last kinetochore, leading to dissociation of the APC/C inhibitor complex. The combination of inhibiting MCC formation and dissociation results in rapid checkpoint inactivation and thus mitotic exit. It is very likely that protein phosphates also play a major role in the checkpoint of higher eukaryotes since it has such an prominent role in yeast. There are indeed indications that certain protein phosphatases are required for checkpoint silencing processes including dynein-dependent stripping and complex dissociation. To better understand how the inhibitor gets inhibited it is important to better understand how the APC/C is activated by its co-activator CDC20 and how checkpoint signaling prevents this. In conclusion, although there are significant advances in checkpoint research field, there still remains a lot to be disentangled.

1. Wheatley, S.P., Henzing, A.J., Dodson, H., Khaled, W. & Earnshaw, W.C. Aurora-B phosphorylation in vitro identifies a residue of survivin that is essential for its localization and binding to inner centromere protein (INCENP) in vivo. *J Biol Chem* **279**, 5655-60 (2004).
2. Bishop, J.D. & Schumacher, J.M. Phosphorylation of the carboxyl terminus of inner centromere protein (INCENP) by the Aurora B Kinase stimulates Aurora B kinase activity. *J Biol Chem* **277**, 27577-80 (2002).
3. Honda, R., Korner, R. & Nigg, E.A. Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. *Mol Biol Cell* **14**, 3325-41 (2003).
4. Sessa, F. et al. Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. *Mol Cell* **18**, 379-91 (2005).
5. Klein, U.R., Nigg, E.A. & Gruneberg, U. Centromere targeting of the chromosomal passenger complex requires a ternary subcomplex of Borealin, Survivin, and the N-terminal domain of INCENP. *Mol Biol Cell* **17**, 2547-58 (2006).
6. Vader, G., Kauw, J.J., Medema, R.H. & Lens, S.M. Survivin mediates targeting of the chromosomal passenger complex to the centromere and midbody. *EMBO Rep* **7**, 85-92 (2006).
7. Jeyaprakash, A.A. et al. Structure of a Survivin-Borealin-INCENP core complex reveals how chromosomal passengers travel together. *Cell* **131**, 271-85 (2007).
8. Gassmann, R. et al. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. *J Cell Biol* **166**, 179-91 (2004).
9. Tanaka, T.U. et al. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell* **108**, 317-29 (2002).
10. Cimini, D., Wan, X., Hirel, C.B. & Salmon, E.D. Aurora kinase promotes turnover of kinetochore microtubules to reduce chromosome segregation errors. *Curr Biol* **16**, 1711-8 (2006).
11. Lampson, M.A., Renduchitala, K., Khodjakov, A. & Kapoor, T.M. Correcting improper chromosome-spindle attachments during cell division. *Nat Cell Biol* **6**, 232-7 (2004).
12. Pinsky, B.A., Kung, C., Shokat, K.M. & Biggins, S. The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. *Nat Cell Biol* **8**, 78-83 (2006).
13. Cheeseman, I.M., Chappie, J.S., Wilson-Kubalek, E.M. & Desai, A. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell* **127**, 983-97 (2006).
14. DeLuca, J.G. et al. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. *Cell* **127**, 969-82 (2006).
15. Ciferri, C. et al. Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. *Cell* **133**, 427-39 (2008).
16. Guimaraes, G.J., Dong, Y., McEwen, B.F. & Deluca, J.G. Kinetochore-microtubule attachment relies on the disordered N-terminal tail domain of Hec1. *Curr Biol* **18**, 1778-84 (2008).
17. Cheeseman, I.M. et al. Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell* **111**, 163-72 (2002).
18. King, J.M. & Nicklas, R.B. Tension on chromosomes increases the number of kinetochore microtubules but only within limits. *J Cell Sci* **113 Pt 21**, 3815-23 (2000).
19. Nicklas, R.B., Waters, J.C., Salmon, E.D. & Ward, S.C. Checkpoint signals in grasshopper meiosis are sensitive to microtubule attachment, but tension is still essential. *J Cell Sci* **114**, 4173-83 (2001).
20. Sampath, S.C. et al. The chromosomal passenger complex is required for chromatin-induced microtubule stabilization and spindle assembly. *Cell* **118**, 187-202 (2004).
21. Liu, D., Vader, G., Vromans, M.J., Lampson, M.A. & Lens, S.M. Sensing chromosome bi-orientation by spatial separation of aurora B kinase from kinetochore substrates. *Science* **323**, 1350-3 (2009).

22. Lindqvist, A., Rodriguez-Bravo, V. & Medema, R.H. The decision to enter mitosis: feedback and redundancy in the mitotic entry network. *J Cell Biol* **185**, 193-202 (2009).
23. Knowlton, A.L., Lan, W.J. & Stukenberg, P.T. Aurora B is enriched at merotelic attachment sites, where it regulates MCAK. *Current Biology* **16**, 1705-1710 (2006).
24. Zhang, X., Lan, W., Ems-McClung, S.C., Stukenberg, P.T. & Walczak, C.E. Aurora B phosphorylates multiple sites on mitotic centromere-associated kinesin to spatially and temporally regulate its function. *Mol Biol Cell* **18**, 3264-76 (2007).
25. Clute, P. & Pines, J. Temporal and spatial control of cyclin B1 destruction in metaphase. *Nat Cell Biol* **1**, 82-7 (1999).
26. Hagting, A. et al. Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1. *J Cell Biol* **157**, 1125-37 (2002).
27. van Leuken, R., Clijsters, L. & Wolthuis, R. To cell cycle, swing the APC/C. *Biochim Biophys Acta* **1786**, 49-59 (2008).
28. Peters, J.M. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol* **7**, 644-56 (2006).
29. Yu, H. Cdc20: a WD40 activator for a cell cycle degradation machine. *Mol Cell* **27**, 3-16 (2007).
30. Pesin, J.A. & Orr-Weaver, T.L. Regulation of APC/C activators in mitosis and meiosis. *Annu Rev Cell Dev Biol* **24**, 475-99 (2008).
31. Holland, A.J. & Cleveland, D.W. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat Rev Mol Cell Biol* **10**, 478-87 (2009).
32. Kops, G.J., Weaver, B.A. & Cleveland, D.W. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat Rev Cancer* **5**, 773-85 (2005).
33. Rieder, C.L., Cole, R.W., Khodjakov, A. & Sluder, G. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J Cell Biol* **130**, 941-8 (1995).
34. Musacchio, A. & Salmon, E.D. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol* **8**, 379-393 (2007).
35. Hoyt, M.A., Totis, L. & Roberts, B.T. S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**, 507-17 (1991).
36. Taylor, S.S., Scott, M.I. & Holland, A.J. The spindle checkpoint: a quality control mechanism which ensures accurate chromosome segregation. *Chromosome Res* **12**, 599-616 (2004).
37. Li, R. & Murray, A.W. Feedback control of mitosis in budding yeast. *Cell* **66**, 519-31 (1991).
38. Sudakin, V., Chan, G.K. & Yen, T.J. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. *J Cell Biol* **154**, 925-36 (2001).
39. Hardwick, K.G., Johnston, R.C., Smith, D.L. & Murray, A.W. MAD3 encodes a novel component of the spindle checkpoint which interacts with Bub3p, Cdc20p, and Mad2p. *J Cell Biol* **148**, 871-82 (2000).
40. Herzog, F. et al. Structure of the anaphase-promoting complex/cyclosome interacting with a mitotic checkpoint complex. *Science* **323**, 1477-81 (2009).
41. Maciejowski, J. et al. Mps1 directs the assembly of Cdc20 inhibitory complexes during interphase and mitosis to control M phase timing and spindle checkpoint signaling. *J Cell Biol* **190**, 89-100 (2010).
42. Sliedrecht, T., Zhang, C., Shokat, K.M. & Kops, G.J. Chemical genetic inhibition of Mps1 in stable human cell lines reveals novel aspects of Mps1 function in mitosis. *PLoS One* **5**, e10251 (2010).
43. Meraldi, P., Draviam, V.M. & Sorger, P.K. Timing and checkpoints in the regulation of mitotic progression. *Dev Cell* **7**, 45-60 (2004).
44. Nilsson, J., Yekezare, M., Minshull, J. & Pines, J. The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nat Cell Biol* **10**, 1411-20 (2008).

45. Kulukian, A., Han, J.S. & Cleveland, D.W. Unattached kinetochores catalyze production of an anaphase inhibitor that requires a Mad2 template to prime Cdc20 for BubR1 binding. *Dev Cell* **16**, 105-17 (2009).
46. Sczaniecka, M. et al. The spindle checkpoint functions of Mad3 and Mad2 depend on a Mad3 KEN box-mediated interaction with Cdc20-anaphase-promoting complex (APC/C). *J Biol Chem* **283**, 23039-47 (2008).
47. Fang, G. Checkpoint protein BubR1 acts synergistically with Mad2 to inhibit anaphase-promoting complex. *Mol Biol Cell* **13**, 755-66 (2002).
48. Tang, Z., Bharadwaj, R., Li, B. & Yu, H. Mad2-Independent inhibition of APCCdc20 by the mitotic checkpoint protein BubR1. *Dev Cell* **1**, 227-37 (2001).
49. Wu, H. et al. p55CDC/hCDC20 is associated with BUBR1 and may be a downstream target of the spindle checkpoint kinase. *Oncogene* **19**, 4557-62 (2000).
50. Chan, G.K.T., Jablonski, S.A., Sudakin, V., Hittle, J.C. & Yen, T.J. Human Bubr1 Is a Mitotic Checkpoint Kinase That Monitors Cenp-E Functions at Kinetochores and Binds the Cyclosome/APC. *The Journal of Cell Biology* **146**, 941-954 (1999).
51. Burton, J.L. & Solomon, M.J. Mad3p, a pseudosubstrate inhibitor of APCCdc20 in the spindle assembly checkpoint. *Genes Dev* **21**, 655-67 (2007).
52. King, E.M., van der Sar, S.J. & Hardwick, K.G. Mad3 KEN boxes mediate both Cdc20 and Mad3 turnover, and are critical for the spindle checkpoint. *PLoS One* **2**, e342 (2007).
53. Rahmani, Z., Gagou, M.E., Lefebvre, C., Emre, D. & Karess, R.E. Separating the spindle, checkpoint, and timer functions of BubR1. *J Cell Biol* **187**, 597-605 (2009).
54. Pan, J. & Chen, R.H. Spindle checkpoint regulates Cdc20p stability in Saccharomyces cerevisiae. *Genes Dev* **18**, 1439-51 (2004).
55. Chen, R.H., Brady, D.M., Smith, D., Murray, A.W. & Hardwick, K.G. The spindle checkpoint of budding yeast depends on a tight complex between the Mad1 and Mad2 proteins. *Mol Biol Cell* **10**, 2607-18 (1999).
56. Sironi, L. et al. Mad2 binding to Mad1 and Cdc20, rather than oligomerization, is required for the spindle checkpoint. *EMBO J* **20**, 6371-82 (2001).
57. Luo, X., Tang, Z., Rizo, J. & Yu, H. The Mad2 spindle checkpoint protein undergoes similar major conformational changes upon binding to either Mad1 or Cdc20. *Mol Cell* **9**, 59-71 (2002).
58. Luo, X. et al. The Mad2 spindle checkpoint protein has two distinct natively folded states. *Nat Struct Mol Biol* **11**, 338-45 (2004).
59. Hwang, L.H. et al. Budding yeast Cdc20: a target of the spindle checkpoint. *Science* **279**, 1041-4 (1998).
60. De Antoni, A. et al. The Mad1/Mad2 complex as a template for Mad2 activation in the spindle assembly checkpoint. *Curr Biol* **15**, 214-25 (2005).
61. Howell, B.J. et al. Spindle checkpoint protein dynamics at kinetochores in living cells. *Curr Biol* **14**, 953-64 (2004).
62. Sironi, L. et al. Crystal structure of the tetrameric Mad1-Mad2 core complex: implications of a 'safety belt' binding mechanism for the spindle checkpoint. *EMBO J* **21**, 2496-506 (2002).
63. Vink, M. et al. In vitro FRAP identifies the minimal requirements for Mad2 kinetochore dynamics. *Curr Biol* **16**, 755-66 (2006).
64. Yang, M. et al. Insights into mad2 regulation in the spindle checkpoint revealed by the crystal structure of the symmetric mad2 dimer. *PLoS Biol* **6**, e50 (2008).
65. Lad, L., Lichtsteiner, S., Hartman, J.J., Wood, K.W. & Sakowicz, R. Kinetic analysis of Mad2-Cdc20 formation: conformational changes in Mad2 are catalyzed by a C-Mad2-ligand complex. *Biochemistry* **48**, 9503-15 (2009).
66. Simonetta, M. et al. The influence of catalysis on mad2 activation dynamics. *PLoS Biol* **7**, e10 (2009).

67. Mapelli, M., Massimiliano, L., Santaguida, S. & Musacchio, A. The Mad2 conformational dimer: structure and implications for the spindle assembly checkpoint. *Cell* **131**, 730-43 (2007).
68. Luo, X. et al. Structure of the Mad2 spindle assembly checkpoint protein and its interaction with Cdc20. *Nat Struct Biol* **7**, 224-9 (2000).
69. Kops, G.J. et al. ZW10 links mitotic checkpoint signaling to the structural kinetochore. *J Cell Biol* **169**, 49-60 (2005).
70. Buffin, E., Lefebvre, C., Huang, J., Gagou, M.E. & Karess, R.E. Recruitment of Mad2 to the kinetochore requires the Rod/Zw10 complex. *Curr Biol* **15**, 856-61 (2005).
71. Abrieu, A. et al. Mps1 is a kinetochore-associated kinase essential for the vertebrate mitotic checkpoint. *Cell* **106**, 83-93 (2001).
72. Liu, S.T. et al. Human MPS1 kinase is required for mitotic arrest induced by the loss of CENP-E from kinetochores. *Mol Biol Cell* **14**, 1638-51 (2003).
73. Tighe, A., Staples, O. & Taylor, S. Mps1 kinase activity restrains anaphase during an unperturbed mitosis and targets Mad2 to kinetochores. *J Cell Biol* **181**, 893-901 (2008).
74. Kwiatkowski, N. et al. Small-molecule kinase inhibitors provide insight into Mps1 cell cycle function. *Nat Chem Biol* **6**, 359-68 (2010).
75. Santaguida, S., Tighe, A., D'Alise, A.M., Taylor, S.S. & Musacchio, A. Dissecting the role of MPS1 in chromosome biorientation and the spindle checkpoint through the small molecule inhibitor reversine. *J Cell Biol* **190**, 73-87 (2010).
76. Stucke, V.M., Baumann, C. & Nigg, E.A. Kinetochore localization and microtubule interaction of the human spindle checkpoint kinase Mps1. *Chromosoma* **113**, 1-15 (2004).
77. Lauze, E. et al. Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. *EMBO J* **14**, 1655-63 (1995).
78. Hewitt, L. et al. Sustained Mps1 activity is required in mitosis to recruit O-Mad2 to the Mad1-C-Mad2 core complex. *J Cell Biol* **190**, 25-34 (2010).
79. Kallio, M.J., McCleland, M.L., Stukenberg, P.T. & Gorbsky, G.J. Inhibition of aurora B kinase blocks chromosome segregation, overrides the spindle checkpoint, and perturbs microtubule dynamics in mitosis. *Curr Biol* **12**, 900-5 (2002).
80. Petersen, J. & Hagan, I.M. S. pombe aurora kinase/survivin is required for chromosome condensation and the spindle checkpoint attachment response. *Curr Biol* **13**, 590-7 (2003).
81. Vanoosthuyse, V. & Hardwick, K.G. A novel protein phosphatase 1-dependent spindle checkpoint silencing mechanism. *Curr Biol* **19**, 1176-81 (2009).
82. Tang, Z., Shu, H., Oncel, D., Chen, S. & Yu, H. Phosphorylation of Cdc20 by Bub1 provides a catalytic mechanism for APC/C inhibition by the spindle checkpoint. *Mol Cell* **16**, 387-97 (2004).
83. Yudkovsky, Y., Shteinberg, M., Listovsky, T., Brandeis, M. & Hershko, A. Phosphorylation of Cdc20/fizzy negatively regulates the mammalian cyclosome/APC in the mitotic checkpoint. *Biochem Biophys Res Commun* **271**, 299-304 (2000).
84. Chung, E. & Chen, R.H. Phosphorylation of Cdc20 is required for its inhibition by the spindle checkpoint. *Nat Cell Biol* **5**, 748-53 (2003).
85. Kang, J. et al. Structure and substrate recruitment of the human spindle checkpoint kinase Bub1. *Mol Cell* **32**, 394-405 (2008).
86. Rieder, C.L., Schultz, A., Cole, R. & Sluder, G. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J Cell Biol* **127**, 1301-10 (1994).
87. Maresca, T.J. & Salmon, E.D. Welcome to a new kind of tension: translating kinetochore mechanics into a wait-anaphase signal. *J Cell Sci* **123**, 825-35 (2010).
88. Khodjakov, A. & Pines, J. Centromere tension: a divisive issue. *Nat Cell Biol* **12**, 919-23 (2010).
89. Nezi, L. & Musacchio, A. Sister chromatid tension and the spindle assembly checkpoint. *Curr Opin Cell Biol* **21**, 785-95 (2009).

90. O'Connell, C.B. et al. The spindle assembly checkpoint is satisfied in the absence of interkinetochore tension during mitosis with unreplicated genomes. *J Cell Biol* **183**, 29-36 (2008).
91. Stern, B.M. & Murray, A.W. Lack of tension at kinetochores activates the spindle checkpoint in budding yeast. *Curr Biol* **11**, 1462-7 (2001).
92. Li, X. & Nicklas, R.B. Mitotic forces control a cell-cycle checkpoint. *Nature* **373**, 630-2 (1995).
93. Waters, J.C., Chen, R.-H., Murray, A.W. & Salmon, E.D. Localization of Mad2 to Kinetochores Depends on Microtubule Attachment, Not Tension. *The Journal of Cell Biology* **141**, 1181-1191 (1998).
94. Yang, Z., Kenny, A.E., Brito, D.A. & Rieder, C.L. Cells satisfy the mitotic checkpoint in Taxol, and do so faster in concentrations that stabilize syntelic attachments. *J Cell Biol* **186**, 675-84 (2009).
95. Uchida, K.S. et al. Kinetochore stretching inactivates the spindle assembly checkpoint. *J Cell Biol* **184**, 383-90 (2009).
96. Maresca, T.J. & Salmon, E.D. Intrakinetochore stretch is associated with changes in kinetochore phosphorylation and spindle assembly checkpoint activity. *J Cell Biol* **184**, 373-81 (2009).
97. Habu, T., Kim, S.H., Weinstein, J. & Matsumoto, T. Identification of a MAD2-binding protein, CMT2, and its role in mitosis. *EMBO J* **21**, 6419-6428 (2002).
98. Xia, G. et al. Conformation-specific binding of p31comet antagonizes the function of Mad2 in the spindle checkpoint. *EMBO J* **23**, 3133-3143 (2004).
99. Mapelli, M. et al. Determinants of conformational dimerization of Mad2 and its inhibition by p31comet. *EMBO J* **25**, 1273-1284 (2006).
100. Yang, M. et al. p31comet blocks Mad2 activation through structural mimicry. *Cell* **131**, 744-55 (2007).
101. Ibrahim, B., Dittrich, P., Diekmann, S. & Schmitt, E. Mad2 binding is not sufficient for complete Cdc20 sequestering in mitotic transition control (an in silico study). *Biophys Chem* **134**, 93-100 (2008).
102. Abrieu, A., Kahana, J.A., Wood, K.W. & Cleveland, D.W. CENP-E as an essential component of the mitotic checkpoint in vitro. *Cell* **102**, 817-26 (2000).
103. Mao, Y., Abrieu, A. & Cleveland, D.W. Activating and silencing the mitotic checkpoint through CENP-E-dependent activation/inactivation of BubR1. *Cell* **114**, 87-98 (2003).
104. Mao, Y., Desai, A. & Cleveland, D.W. Microtubule capture by CENP-E silences BubR1-dependent mitotic checkpoint signaling. *The Journal of Cell Biology* **170**, 873-880 (2005).
105. Weaver, B.A. et al. Centromere-associated protein-E is essential for the mammalian mitotic checkpoint to prevent aneuploidy due to single chromosome loss. *J Cell Biol* **162**, 551-63 (2003).
106. Malureanu, L.A. et al. BubR1 N terminus acts as a soluble inhibitor of cyclin B degradation by APC/C(Cdc20) in interphase. *Dev Cell* **16**, 118-31 (2009).
107. Elowe, S. et al. Uncoupling of the spindle-checkpoint and chromosome-congression functions of BubR1. *J Cell Sci* **123**, 84-94 (2010).
108. Maia, A.F., Feijao, T., Vromans, M.J., Sunkel, C.E. & Lens, S.M. Aurora B kinase cooperates with CENP-E to promote timely anaphase onset. *Chromosoma* **119**, 405-13 (2010).
109. Tanudji, M. et al. Gene silencing of CENP-E by small interfering RNA in HeLa cells leads to missegregation of chromosomes after a mitotic delay. *Mol Biol Cell* **15**, 3771-81 (2004).
110. Lampson, M.A. & Kapoor, T.M. The human mitotic checkpoint protein BubR1 regulates chromosome-spindle attachments. *Nat Cell Biol* **7**, 93-8 (2005).
111. Elowe, S., Hummer, S., Uldschmid, A., Li, X. & Nigg, E.A. Tension-sensitive Plk1 phosphorylation on BubR1 regulates the stability of kinetochore microtubule interactions. *Genes Dev* **21**, 2205-19 (2007).
112. Matsumura, S., Toyoshima, F. & Nishida, E. Polo-like kinase 1 facilitates chromosome alignment during prometaphase through BubR1. *J Biol Chem* **282**, 15217-27 (2007).

113. Huang, H. et al. Phosphorylation sites in BubR1 that regulate kinetochore attachment, tension, and mitotic exit. *J Cell Biol* **183**, 667-80 (2008).
114. Kang, J., Chen, Y., Zhao, Y. & Yu, H. Autophosphorylation-dependent activation of human Mps1 is required for the spindle checkpoint. *Proc Natl Acad Sci U S A* **104**, 20232-7 (2007).
115. Mattison, C.P. et al. Mps1 activation loop autophosphorylation enhances kinase activity. *J Biol Chem* **282**, 30553-61 (2007).
116. Jelluma, N. et al. Chromosomal instability by inefficient Mps1 auto-activation due to a weakened mitotic checkpoint and lagging chromosomes. *PLoS One* **3**, e2415 (2008).
117. Wang, W. et al. Structural and mechanistic insights into Mps1 kinase activation. *J Cell Mol Med* **13**, 1679-94 (2009).
118. Zhao, Y. & Chen, R.H. Mps1 phosphorylation by MAP kinase is required for kinetochore localization of spindle-checkpoint proteins. *Curr Biol* **16**, 1764-9 (2006).
119. Xu, Q. et al. Regulation of kinetochore recruitment of two essential mitotic spindle checkpoint proteins by Mps1 phosphorylation. *Mol Biol Cell* **20**, 10-20 (2009).
120. Yamaguchi, S., Decottignies, A. & Nurse, P. Function of Cdc2p-dependent Bub1p phosphorylation and Bub1p kinase activity in the mitotic and meiotic spindle checkpoint. *EMBO J* **22**, 1075-87 (2003).
121. Chen, R.H. Phosphorylation and activation of Bub1 on unattached chromosomes facilitate the spindle checkpoint. *EMBO J* **23**, 3113-21 (2004).
122. Pinsky, B.A., Nelson, C.R. & Biggins, S. Protein phosphatase 1 regulates exit from the spindle checkpoint in budding yeast. *Curr Biol* **19**, 1182-7 (2009).
123. Emanuele, M.J. et al. Aurora B kinase and protein phosphatase 1 have opposing roles in modulating kinetochore assembly. *J Cell Biol* **181**, 241-54 (2008).
124. Akiyoshi, B., Nelson, C.R., Ranish, J.A. & Biggins, S. Quantitative proteomic analysis of purified yeast kinetochores identifies a PP1 regulatory subunit. *Genes Dev* **23**, 2887-99 (2009).
125. Wassmann, K., Liberal, V. & Benezra, R. Mad2 phosphorylation regulates its association with Mad1 and the APC/C. *EMBO J* **22**, 797-806 (2003).
126. Hoffman, D.B., Pearson, C.G., Yen, T.J., Howell, B.J. & Salmon, E.D. Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at PtK1 kinetochores. *Mol Biol Cell* **12**, 1995-2009 (2001).
127. Shah, J.V. et al. Dynamics of centromere and kinetochore proteins; implications for checkpoint signaling and silencing. *Curr Biol* **14**, 942-52 (2004).
128. Howell, B.J. et al. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *The Journal of Cell Biology* **155**, 1159-1172 (2001).
129. Whyte, J. et al. Phosphorylation regulates targeting of cytoplasmic dynein to kinetochores during mitosis. *J Cell Biol* **183**, 819-34 (2008).
130. Griffis, E.R., Stuurman, N. & Vale, R.D. Spindly, a novel protein essential for silencing the spindle assembly checkpoint, recruits dynein to the kinetochore. *The Journal of Cell Biology* **177**, 1005-1015 (2007).
131. Gassmann, R. et al. A new mechanism controlling kinetochore-microtubule interactions revealed by comparison of two dynein-targeting components: SPDL-1 and the Rod/Zwilch/Zw10 complex. *Genes Dev* **22**, 2385-99 (2008).
132. Chan, Y.W. et al. Mitotic control of kinetochore-associated dynein and spindle orientation by human Spindly. *The Journal of Cell Biology* **185**, 859-874 (2009).
133. Gassmann, R. et al. Removal of Spindly from microtubule-attached kinetochores controls spindle checkpoint silencing in human cells. *Genes & Development* **24**, 957-971 (2010).
134. Jelluma, N., Dansen, T.B., Sliedrecht, T., Kwiatkowski, N.P. & Kops, G.J.P.L. Release of Mps1 from kinetochores is crucial for timely anaphase onset. *The Journal of Cell Biology* **191**, 281-290 (2010).
135. Palframan, W.J., Meehl, J.B., Jaspersen, S.L., Winey, M. & Murray, A.W. Anaphase inactivation of the spindle checkpoint. *Science* **313**, 680-4 (2006).

136. Miniowitz-Shemtov, S., Teichner, A., Sitry-Shevah, D. & Hershko, A. ATP is required for the release of the anaphase-promoting complex/cyclosome from inhibition by the mitotic checkpoint. *Proc Natl Acad Sci U S A* **107**, 5351-6 (2010).
137. Reddy, S.K., Rape, M., Margansky, W.A. & Kirschner, M.W. Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* **446**, 921-5 (2007).
138. Stegmeier, F. et al. Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* **446**, 876-81 (2007).
139. Visconti, R., Palazzo, L. & Grieco, D. Requirement for proteolysis in spindle assembly checkpoint silencing. *Cell Cycle* **9**, 564-9 (2010).
140. Choi, E. et al. BubR1 acetylation at prometaphase is required for modulating APC/C activity and timing of mitosis. *EMBO J* **28**, 2077-89 (2009).
141. Rudner, A.D. & Murray, A.W. Phosphorylation by Cdc28 activates the Cdc20-dependent activity of the anaphase-promoting complex. *J Cell Biol* **149**, 1377-90 (2000).
142. Kraft, C. et al. Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *EMBO J* **22**, 6598-609 (2003).