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Mutual regulation between the spindle checkpoint and APC/C

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Abstract

Accurate chromosome segregation during mitosis is critical for maintaining genomic stability. The spindle checkpoint is a cellular surveillance system that ensures the fidelity of chromosome segregation. In response to sister chromatids not properly captured by spindle microtubules, the spindle checkpoint interferes with the functions of Cdc20, the mitotic activator of the anaphase-promoting complex or cyclosome (APC/C), thereby blocking APC/C-mediated degradation of securin and cyclin B to delay anaphase onset. This review summarizes the recent progress in the mechanisms by which checkpoint proteins inhibit APC/C, the conformational and enzymatic activation of checkpoint proteins, and the emerging roles of APC/C-dependent ubiquitination in checkpoint inactivation.

Keywords

Mitosis; Chromosome Segregation; Cell Cycle; Ubiquitination

1. Introduction

The cell division cycle consists of a series of temporally and spatially regulated events. Certain cell cycle processes need to be completed before others are initiated. For example, DNA replication needs to be completed before chromosome segregation occurs. The periodic rise and fall of the cyclin-dependent kinase (Cdk) activities are critical for the orderly progression of the cell cycle. An important mechanism that regulates the Cdk activities is timely degradation of cyclins and Cdk inhibitors through the ubiquitin-proteasome pathway.

The anaphase-promoting complex or cyclosome (APC/C) is a multisubunit ubiquitin ligase that regulates multiple cell cycle transitions, including the metaphase–anaphase transition and exit from mitosis [1–3]. Two APC/C activators, Cdc20 and Cdh1, directly bind to APC/C, activate its ubiquitin ligase activity, and contribute to its substrate recognition and specificity. Cdc20 is the mitotic activator of APC/C while Cdh1 mainly interacts with APC/C in telophase and G1. Securin and mitotic cyclins are the major substrates of APC/C^{Cdc20} during mitosis. Degradation of securin and cyclin B activates separase, which cleaves the cohesin complex and triggers sister-chromatid separation. Cyclin B degradation also inactivates Cdk1 and promotes mitotic exit (Fig. 1).

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The preservation of genome integrity is a critical task of cell division. To accomplish this task, cells duplicate their chromosomes during S phase and hold sister chromosomes together through sister-chromatid cohesion. In mitosis, the spindle checkpoint monitors the microtubule attachment to and the tension between the two opposing kinetochores of all pairs of sister chromatids [4,5] (Fig. 1). A single unattached kinetochore in a mammalian cell is sufficient to activate the spindle checkpoint, which blocks APC/C^{Cdc20} activation and anaphase onset. Only when all pairs of sister kinetochores are properly captured by spindle microtubules and are under tension, APC/C^{Cdc20} is activated, leading to the degradation of securin and cyclin B, activation of separase, removal of sister-chromatid cohesion, and sister-chromatid separation. In this review, we discuss recent findings that have advanced our understanding of spindle checkpoint activation and inactivation, and highlight the mutual antagonism between the spindle checkpoint and its major target APC/C^{Cdc20}.

2. Inhibition of APC/C^{Cdc20} by the spindle checkpoint

The core components of the spindle checkpoint include the mitotic arrest deficiency (Mad) 1–3 and budding uninhibited by benomyl (Bub) 1–3 proteins. They were initially discovered through genetic studies in the budding yeast and were later shown to be conserved from yeast to man [4,5]. Upon checkpoint activation, these proteins use multiple strategies to inhibit APC/C^{Cdc20} upon checkpoint activation, including the stoichiometric binding to and sequestration of Cdc20 by Mad2 and BubR1, and phosphorylation of Cdc20 by Bub1 [3] (Fig. 2).

2.1. Mechanism of APC/C-dependent ubiquitination

To review how the spindle checkpoint inhibits APC/C, we briefly summarize how APC/C ubiquitinates its substrates. APC/C is a multisubunit ubiquitin ligase distantly related to the cullin-RING family of ubiquitin ligases. The subcomplex of Apc2 (a cullin-like subunit) and Apc11 (a RING-containing subunit) recruits and activates ubiquitin-conjugating enzymes (UBCs) [6]. Cdc20 or Cdh1 helps to recruit substrates to APC/C for efficient ubiquitination [1–3]. All APC/C substrates contain small peptide motifs that are critical for their recognition by APC/C, often referred to as APC/C degrons. The destruction box (D-box, with a consensus of RXXLXXXXN) and the KEN-box (with a consensus of KEN) are two well-characterized APC/C degrons. Cdc20 and Cdh1 contain a C-terminal WD40 domain, which has been shown to contact D-box directly through chemical crosslinking [7]. In addition to Cdc20 or Cdh1, Apc10 has been implicated in D-box recognition [8,9]. Recent electron microscopy (EM) studies confirm and extend these previous findings, and show that the D-box binds at the interface formed by Cdh1 and Apc10 [10–12]. How the KEN-box is recognized remains unclear. It is also unclear why many APC/C substrates contain both D- and KEN-boxes.

2.2. Inhibition of Cdc20 by Mad2 and BubR1

Mad2 and BubR1 (the vertebrate homolog of yeast Mad3) directly bind to Cdc20 and inhibit APC/C^{Cdc20} *in vitro* [13,14]. Mad2 and BubR1 together inhibit APC/C with greater efficiency than either alone does [15]. Indeed, Mad2 and BubR1 are parts of a larger mitotic checkpoint complex (MCC) that contains Mad2, BubR1, Bub3, and Cdc20, and inhibit APC/C^{Cdc20} synergistically *in vivo* [16,17]. On the other hand, depletion of either Mad2 or BubR1 from human cells by RNA interference (RNAi) accelerates mitotic progression and shortens the mitotic duration between nuclear envelope breakdown and anaphase onset [18]. Depletion of both has a greater effect than depletion of either alone, suggesting that Mad2 and BubR1 might have residual APC/C-inhibitory activities in the absence of each other.

2.3. Conformational activation of Mad2

Mouse genetic studies have demonstrated the physiological importance of the Mad2–Cdc20 interaction in maintaining chromosomal stability [19]. Consistently, binding of Mad2 to Cdc20 is tightly regulated by the spindle checkpoint. Efforts to understand this regulation have led to the discovery that Mad2 is an unusual two-state protein [13,20–26]. Mad2 has two native folds: a latent open conformer (O-Mad2; also known as N1-Mad2) and a closed conformer (C-Mad2; also known as N2-Mad2) that is more active in Cdc20 binding and APC/C inhibition. The current available evidence is consistent with the following model for the conformational activation of Mad2 [27,28] (Fig. 2). Mad2 binds to its upstream regulator Mad1 throughout the cell cycle, forming a tight Mad1–C-Mad2 core complex. Upon spindle checkpoint activation, the Mad1–Mad2 core complex is targeted to unattached kinetochores, and further recruits cytosolic O-Mad2 through O–C Mad2 dimerization. The asymmetric O–C Mad2 dimerization induces a conformational change of O-Mad2, which adopts a transient intermediate conformation (I-Mad2). I-Mad2 can complete the O–C conformational activation and dissociate from the Mad1–Mad2 core to become C-Mad2 without a ligand, which then binds Cdc20. Alternatively, I-Mad2 is directly captured by kinetochore-bound Cdc20, forming the Mad2–Cdc20 complex. How Mad2 binding to Cdc20 inhibits APC/C is not understood.

2.4. BubR1/Mad3 as a pseudo-substrate inhibitor of APC/C^{Cdc20}

BubR1 inhibits the activity of APC/C^{Cdc20} more effectively than Mad2 does *in vitro* [14,15]. BubR1 contains an N-terminal tetratricopeptide repeat (TPR) domain required for kinetochore targeting, a conserved GLEBS motif that mediates Bub3 binding, and a C-terminal kinase domain. The kinase domain of BubR1 is not conserved in yeast and is not required for APC/C inhibition. Many studies have been devoted to dissect the functions of various domains of BubR1 and to understand the mechanism by which BubR1 inhibits APC/C^{Cdc20} [29–35].

Yeast Mad3 contains KEN boxes, which are required for Mad3 binding to Cdc20 and for the spindle checkpoint function of Mad3 [29–31]. Furthermore, the KEN boxes of Mad3 compete with KEN-box-containing APC/C substrates for binding to Cdc20. The requirement for the KEN box of BubR1 in Cdc20 binding and in the spindle checkpoint is conserved in higher organisms, including human, mouse, and fly [32,33,35]. Interestingly, BubR1/Mad3 itself does not undergo APC/C^{Cdc20}-dependent degradation. Therefore, these results suggest that BubR1/Mad3 acts as a competitive, pseudo-substrate inhibitor of APC/C^{Cdc20}.

These findings raise two unresolved questions. First, why is BubR1 not ubiquitinated by APC/C^{Cdc20} and degraded? Acetylation of BubR1 at K250 by the acetyltransferase PCAF has been suggested to allow BubR1 to escape APC/C^{Cdc20}-dependent ubiquitination [36]. The underlying mechanism of how acetylation prevents BubR1 ubiquitination is not established, however. Second, two major APC/C^{Cdc20} substrates, cyclin B and securin, contain a D-box. If BubR1 uses its KEN-box to compete with substrates for binding to APC/C^{Cdc20}, how does it inhibit D-box-dependent ubiquitination? Along this vein, several studies have shown that human BubR1 contains other Cdc20-binding motifs, in addition to the KEN-boxes [14,35,37]. It will be interesting to test whether these BubR1 motifs block D-box-dependent ubiquitination by APC/C^{Cdc20}.

2.5. Inhibition of APC/C by MCC

Although BubR1 and Mad2 can independently inhibit APC/C^{Cdc20} *in vitro*, they most likely collaborate with each other to inhibit APC/C^{Cdc20} in cells by forming MCC. By using purified chromosomes from mitotic arrested HeLa extract, Kulukian *et al.* have investigated the role of unattached kinetochores in MCC generation and APC/C^{Cdc20} inhibition [38].

Unattached kinetochores enhance inhibition of APC/C by purified MCC components (Mad2, BubR1, and Bub3) and binding of BubR1 to APC/C^{Cdc20}. This enhancement depends on kinetochore-bound Mad1–Mad2 core complex and Mad2 dimerization. These results suggest that unattached kinetochores produce active Mad2 (C-Mad2) and assemble an initial Mad2–Cdc20 complex to promote further interaction with BubR1–Bub3. Thus, this study provides further support a role of unattached kinetochores in the formation of MCC (Fig. 2).

How does MCC inhibit APC/C^{Cdc20}? Recent EM studies of APC/C bound to Cdh1 or bound to MCC have provided key insights into this question [10–12,39]. It appears that Cdc20 alone and Cdc20 as a component of MCC bind to APC/C in different orientations and at different sites. In other words, binding of BubR1–Bub3 and Mad2 to Cdc20 alters the interaction between Cdc20 and APC/C. In doing so, they may compromise the substrate-recognition site formed between Cdc20 and Apc10. Therefore, MCC inhibits the activity of APC/C^{Cdc20} by blocking productive substrate binding both competitively and non-competitively.

2.6. Inhibition of APC/C by Cdc20 phosphorylation

Cdc20 can be phosphorylated by Bub1, MAPK, and Cdk1 [40–42]. Phosphorylation of Cdc20 at S153 by Bub1 has been shown to inhibit the activity of APC/C^{Cdc20} [40]. Similar to BubR1, Bub1 contains KEN-boxes that interact with Cdc20 [43]. KEN-box-dependent binding then recruits Cdc20 to Bub1 and enables efficient phosphorylation of Cdc20 by Bub1. Bub1 is phosphorylated in mitosis, and the chromosome-bound Bub1 is hyperphosphorylated, suggesting that the kinase activity of Bub1 might be enhanced during checkpoint activation [44]. It is unclear how phosphorylation of Cdc20 inhibits APC/C^{Cdc20}. It is also unknown whether and how Bub1-mediated phosphorylation of Cdc20 regulates MCC formation in cells.

3. Kinetochore signaling in the spindle checkpoint

The existence of a single unattached kinetochore within a cell is sufficient to activate spindle checkpoint. The spindle checkpoint proteins are targeted to unattached kinetochores in a hierarchical fashion. Although the kinetochore is a massive protein assembly with more than 90 proteins, the KMN network that consists of Knl1/blinkin, the Ndc80 complex (Ndc80–Nuf2–Spc25–Spc24), and the Mis12 complex (Dsn1–Nsl1–Nnf1–Mis12) has emerged as a major receptor for both microtubules and checkpoint proteins [45] (Fig. 2 & 3).

3.1. Aurora B as a tension sensor

Aurora B as a part of the chromosome passenger complex (CPC; Aurora B–INCENP–survivin–borealin) lies at the top of the hierarchy for targeting checkpoint proteins to kinetochores [46]. Aurora B also promotes proper kinetochore-microtubule attachment by phosphorylating several proteins in the KMN network and thus destabilizing improper microtubule binding to kinetochores [47]. Aurora B is located at the inner centromere. The degree of phosphorylation of Aurora B substrates at the kinetochores is inversely correlated with the distance between Aurora B and its substrates [48]. It has been postulated that tension between an opposing pair of kinetochores may cause deformation of kinetochores and a physical separation between Aurora B and its substrates, thereby influencing the phosphorylation status of key kinetochore and checkpoint proteins. Aurora B can, in principle, act as a sensor for the tension (or lack thereof) across kinetochores.

3.2. Recruitment of Bub1 and BubR1

Bub1 localizes to kinetochores in mitosis and mediates the targeting other mitotic regulators to kinetochores, including BubR1 and Plk1 [49]. Ectopic targeting of Bub1 to telomeres is

sufficient to recruit Bub3 and Mad3 (the yeast counterpart of BubR1) to telomeres in fission yeast [50], suggesting a direct connection between these proteins in their kinetochore targeting. Indeed, it has recently been shown that Bub1 and BubR1 are recruited to kinetochores through their interactions with blinkin/Knl1 [51]. RNAi-mediated depletion of blinkin causes defective Bub1 and BubR1 kinetochore localization while the kinetochore localization of other KMN network components, is unaffected. The N-terminal TPR domains of Bub1 and BubR1 interact directly with adjacent but distinct conserved motifs in the N-terminal region of blinkin [51,52]. Therefore, blinkin is the major receptor of Bub1 and BubR1 at the kinetochores. On the other hand, the kinetochore targeting of Bub1 and BubR1 is dependent on Bub3 [35,53]. The requirement of Bub3 in Bub1 and BubR1 kinetochore targeting is not mechanistically understood.

In addition to its functions in the spindle checkpoint, Bub1 phosphorylates the C-terminal tail of histone H2A and contributes to the recruitment of the Sgo1–PP2A complex to centromeres, where it protects the centromeric pool of cohesin from Plk1- and Wapl-dependent removal [54–62]. It is unclear, however, whether the kinetochore targeting of Bub1 is required for efficient H2A phosphorylation at the centromeres and for the centromere targeting of the Sgo1–PP2A complex.

3.3. Mps1 as a regulator of Mad1 and Mad2 kinetochore targeting

It is well established that Mps1 has a conserved function in regulating the kinetochore localization of Mad1 and Mad2 [63]. Recent studies using chemical inhibitors of Mps1 have revealed additional intricacies in this regulation [64–68]. In particular, Hewitt *et al.* have presented data to suggest that Mps1 regulates O-Mad2 recruitment to the Mad1–Mad2 core complex in mitosis of human cells [64]. By using a chemical inhibitor AZ3146 to inhibit Mps1 during different cell cycle stages, they have shown that inhibition of the Mps1 kinase activity before mitotic entry disrupts the kinetochore localization of both Mad1 and Mad2. However, inhibition of Mps1 after mitotic entry prevents O-Mad2 recruitment to kinetochore, but does not affect the kinetochore localization of the Mad1–Mad2 core complex. Therefore, these findings suggest that the recruitment of cytosolic O-Mad2 to kinetochores and possibly Mad2 conformational activation is regulated by upstream checkpoint signaling and is thus sensitive to checkpoint status. How Mps1 regulates Mad1 and O-Mad2 recruitment to kinetochores is not understood.

How is Mps1 itself targeted to and activated at the kinetochores? The kinetochore targeting of Mps1 requires the Ndc80 complex [69]. Mps1 activation is dependent on autophosphorylation events on its activation loop [70]. Chemically induced dimerization of Mps1 is sufficient to activate Mps1 [70]. Mps1 indeed forms oligomers in mitotic human cells [64]. Therefore, the current evidence suggests that Mps1 undergoes dimerization and trans-autophosphorylation at kinetochores, leading to its activation. Interestingly, inhibition of its kinase activity leads to further accumulation of Mps1 at kinetochores, suggesting the existence of negative feedback regulation of Mps1 [71].

3.4. Kinetochore targeting of APC/C

The target of the spindle checkpoint, APC/C, is recruited to improperly attached kinetochores in several organisms, in a manner dependent on the activities of the spindle checkpoint and Cdk1 [72–74]. Interestingly, a key APC/C substrate, cyclin B, is also localized at unattached kinetochores during prometaphase and is transported to spindle poles after microtubule attachment in a process requiring dynein/dynactin [75]. The kinetochore localization of cyclin B is attenuated in Mad2-depleted cells. Although the functional importance of the kinetochore localization of APC/C and cyclin B remains to be established,

these results raise the intriguing possibility of local inhibition of APC/C at unattached kinetochores by the spindle checkpoint.

4. Spindle checkpoint silencing

After all sister chromatids are properly attached to kinetochores, the spindle checkpoint needs to be inactivated to release its inhibition of APC/C^{Cdc20}, allowing anaphase onset. Several mechanisms have been implicated in spindle checkpoint silencing (Fig. 3 & 4), including dynein-dependent removal of checkpoint proteins from kinetochores, p31^{comet} binding to and inhibition of C-Mad2, Mad2 phosphorylation, and APC/C-dependent ubiquitination and proteolysis.

4.1. Dynein-mediated removal of checkpoint proteins from kinetochores

The dynein/dynactin complex, a minus-end-directed microtubule motor, is required for transporting Mad1 and Mad2 from kinetochores to spindle poles following kinetochore-microtubule attachment [76]. Blocking this transport by microinjection of a dynein antibody causes prolonged metaphase arrest, indicating a crucial role of this process in checkpoint silencing. Recently, growing evidence has implicated the RZZ complex and spindly in regulating this process (Fig. 3).

RZZ is an evolutionarily conserved kinetochore complex consisting of Rod–Zwisch–ZW10 [77]. It is transported from kinetochores to spindle poles during checkpoint silencing. Spindly is another conserved kinetochore protein that regulates the dynein-mediated transport of Rod and Mad2 at metaphase [78–82]. It is also transported from kinetochores to spindle poles in a dynein-dependent manner following kinetochore-microtubule attachment. The kinetochore localization of spindly depends on the RZZ complex. Depletion of Spindly reduces dynein localization at kinetochores. Moreover, a conserved motif in spindly has been shown to be critical for targeting dynein to kinetochores [81,82]. Expression of a spindly mutant with this motif mutated in cells depleted of endogenous spindly prevents the dynein-dependent removal of checkpoint proteins, resulting in defective checkpoint inactivation. Therefore, it appears that spindly bridges the interaction between RZZ and dynein, and is critical for checkpoint inactivation (Fig. 3).

4.2. p31^{comet} binding to C-Mad2

The Mad2-binding protein, p31^{comet} (initially called Cmt2), adopts a tertiary fold similar to that of Mad2 [83,84]. It selectively binds to the active C-Mad2 conformer and blocks its function in two ways [84–86]. First, it binds to the Mad1–Mad2 core complex and prevents the further recruitment and activation of O-Mad2 (Fig. 3). Second, it binds to Cdc20-bound C-Mad2 and neutralizes the Mad2-inhibitory effects on APC/C^{Cdc20}. Consistent with its Mad2-inhibitory activity, overexpression of p31^{comet} causes mitotic exit in presence of the spindle poison, nocodazole. Conversely, depletion of p31^{comet} delays mitotic exit during the recovery from nocodazole-induced mitotic arrest. Therefore, p31^{comet} is critical for checkpoint silencing. How p31^{comet} blocks the APC/C^{Cdc20}-inhibitory activity of Mad2 without disrupting the Mad2–Cdc20 interaction is not understood.

4.3. Phosphorylation of Mad2

Mad2 is phosphorylated on multiple sites in its C-terminal region [87]. Phosphorylation appears to inhibit Mad2 function, as Mad2 mutants with multiple phosphorylatable residues mutated to aspartates cannot bind to Mad1 or Cdc20. In a recent study, we have further characterized the effects of Mad2 phosphorylation [88]. We show that phosphorylation of Mad2 at a single site, S195, hinders its O–C conformational change. Consistently, the phospho-mimicking Mad2^{S195D} mutant fails to bind to Cdc20, a low-affinity ligand.

Interestingly, Mad2^{S195D} still binds to high-affinity ligands, such as Mad1 and MBP1 (an artificial Mad2-binding peptide), forming ligand-bound C-Mad2. Mad2^{S195D} overexpression causes spindle checkpoint defects in human cells. Mad2^{S195D} appears to bind to the Mad1–Mad2 core complex with higher affinity, forming a stable Mad1–Mad2–Mad2 complex. Thus, we speculate that phosphorylation of Mad2 at S195 may prevent unscheduled activation of Mad2. Alternatively, it may contribute to checkpoint inactivation by capping the Mad1–Mad2 complex and blocking further conformational activation of Mad2, a function that is analogous to that of p31^{comet}. The kinase responsible for phosphorylating Mad2 S195 is unknown. Given the function of Mps1 in recruiting O-Mad2 to the Mad1–Mad2 core complex at the kinetochores, it will be interesting to test whether Mps1 phosphorylates this site on Mad2.

In the same study [88], we have discovered a peculiar effect of the monomeric mutant of Mad2, Mad2^{R133E,Q134A}. We have shown previously that overexpression of another monomeric Mad2 mutant, Mad2^{R133A}, in HeLa cells causes mitotic arrest in the absence of spindle damage, indicating Mad2 dimerization per se is not required for APC/C^{Cdc20} inhibition [22]. By contrast, overexpression of Mad2^{R133E,Q134A} in human cells not only does not cause mitotic arrest, but also inhibits the functions of the endogenous Mad2 in a dominant-negative manner [88]. Because Mad2^{R133E,Q134A} retains its ability to bind to Cdc20, this result suggests that the Mad2 dimerization helix interacts with a yet unidentified protein in cells, and this interaction is critical for APC/C^{Cdc20} inhibition.

4.4. APC/C-dependent Cdc20 ubiquitination

APC/C is the molecular target of the spindle checkpoint. Conversely, APC/C targets several spindle checkpoint proteins for degradation, including Mps1 and Bub1 [89–91]. A recent study has implicated APC/C-dependent ubiquitination in checkpoint silencing [92]. In this study, Zeng *et al.* discover a small molecule called TAME, which prevents APC/C activation by Cdc20 and Cdh1. TAME causes mitotic arrest in human cells. Paradoxically, this mitotic arrest is dependent on the spindle checkpoint, as Mad2 depletion or Aurora B inhibition alleviates TAME-mediated mitotic arrest. A possible explanation for this finding is that partial inhibition of APC/C prevents checkpoint inactivation, but allows cyclin B degradation and mitotic exit in the absence of checkpoint signaling.

The targets of APC/C in checkpoint inactivation remain elusive. Artificial elevation of Mps1 levels or activity can prevent mitotic exit [89], although it remains to be demonstrated whether a non-degradable form of Mps1 delays mitotic exit. A non-degradable mutant of Bub1 with its KEN-boxes mutated does not delay anaphase onset and mitotic exit [90]. A caveat of this study is that the KEN-boxes of Bub1 are also required for efficient Cdc20 phosphorylation [43]. The non-degradable Bub1 mutant is also partially defective in its checkpoint function. Finally, Cdc20 itself undergoes autoubiquitination [93] (Fig. 4). There are conflicting results on the functions of Cdc20 ubiquitination.

In yeast, Cdc20 autoubiquitination and degradation require Mad2 and Mad3 binding, and have been proposed as an APC/C^{Cdc20}-inhibitory checkpoint mechanism [93]. As discussed above, EM studies suggest that Cdc20 in complex with checkpoint proteins binds to different sites on APC/C, as compared to Cdc20 alone. It is possible that the repositioning of Cdc20 caused by Mad2 and Mad3 binding blocks ubiquitination of securin and cyclin B, but favors Cdc20 autoubiquitination.

Cdc20 autoubiquitination is a conserved process in mammalian cells, and is stimulated by p31^{comet} and Ubch10 [94,95]. In two related studies, Cdc20 ubiquitination is reported to cause dissociation of Mad2 from Cdc20 [94,95] (Fig. 4). Moreover, Usp44, a ubiquitin isopeptidase, has been proposed to prevent the autoubiquitination of Cdc20 and maintain the

Mad2–Cdc20 interaction during checkpoint activation. Thus, these two studies implicate Cdc20 autoubiquitination as an important mechanism for checkpoint inactivation.

In a later study, Nilsson *et al.* have constructed a lysine-less Cdc20 mutant that cannot be ubiquitinated [96]. They show that this mutant still dissociates from Mad2 when the checkpoint is inactivated, suggesting that Cdc20 autoubiquitination is not the sole mechanism to dissociate Mad2 from Cdc20. Interestingly, ectopic expression of the lysine-less Cdc20 mutant, even to low levels, causes checkpoint defects. It then appears that Cdc20 ubiquitination might be required for the establishment of the spindle checkpoint, although the underlying mechanism is unclear. Further studies are needed to resolve these apparent discrepancies with respect to Cdc20 autoubiquitination and to uncover the molecular target(s) of APC/C during checkpoint inactivation.

5. Concluding Remarks

Impressive progress has been made towards understanding the molecular mechanisms of the spindle checkpoint. The spindle checkpoint uses an intricate intracellular signal transduction network to sense the existence of unattached or untense kinetochores in the cell, and to block the activity of APC/C^{Cdc20}. Checkpoint proteins are targeted to kinetochores in a hierarchical fashion, leading to their conformational or enzymatic activation. The activated checkpoint proteins then inhibit APC/C^{Cdc20} through multiple mechanisms. Upon proper microtubule attachment at all kinetochores, the checkpoint is inactivated to allow APC/C^{Cdc20} activation and anaphase onset. APC/C^{Cdc20}-mediated ubiquitination has emerged as a major mechanism for checkpoint inactivation. The mutual antagonism between APC/C and the spindle checkpoint produces negative feedback regulation in this system and allows cells to respond rapidly to the status of kinetochore-microtubule attachment.

Many significant questions in the spindle checkpoint remain unanswered, however. How does APC/C recognize substrates at the atomic level? How do Mad2 and BubR1 synergistically bind to and inhibit Cdc20? What are the kinetochore receptors for Mps1 and Mad1? What are the molecular target(s) of APC/C^{Cdc20} in checkpoint inactivation? Future studies aimed at answering these questions will deepen our understanding of this fascinating cellular system that ensures the fidelity of chromosome segregation.

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Fig. 1.

The spindle checkpoint. The anaphase-promoting complex or cyclosome (APC/C) is regulated by the spindle checkpoint during mitosis. During prometaphase, improperly attached kinetochores (red dots) turn on the spindle checkpoint and produce signals to inhibit APC/C^{Cdc20}. After all pairs of sister kinetochores (green dots) are properly attached to spindle microtubules at metaphase, the spindle checkpoint is turned off, and APC/C^{Cdc20} is activated. Active APC/C^{Cdc20} mediates the ubiquitination and degradation of securin and cyclin B, which in turn leads to separase activation, Cdk1 inactivation, and chromosome segregation and mitotic exit.

Fig. 2.

Generation of APC/C-inhibitory checkpoint signals by unattached kinetochores. Mad2 and BubR1 directly bind to Cdc20, forming a larger mitotic checkpoint complex (MCC; BubR1–Bub3–Cdc20–Mad2) to inhibit APC/C^{Cdc20}. Unattached kinetochores recruit and activate Mad2 and BubR1 to promote MCC assembly. The KMN (Knl1–Mis12–Ndc80) network of kinetochore proteins provides a major platform for the recruitment of checkpoint proteins, including Bub1 and BubR1. BubR1 and Bub1 bind to Cdc20 through conserved KEN-boxes (indicated as black bars) and other motifs. The kinetochore targeting of the Mad1–Mad2 core complex also depends on Mps1 and Ndc80. Kinetochore-bound Mad1–Mad2 catalyzes O–C conformational activation of Mad2. MCC binds to and inhibits APC/C^{Cdc20} through blocking substrate recognition. Bub1 phosphorylates Cdc20 and contributes APC/C^{Cdc20} inhibition.

Fig. 3. Spindle checkpoint inactivation by dynein- and microtubule-dependent removal of Mad1–Mad2 from kinetochores and by p31^{comet} binding to C-Mad2. Dynein–dynactin is recruited to properly attached kinetochores by RZZ and spindly, and mediates the poleward transport of Mad1–Mad2. p31^{comet} binds to C-Mad2 in the Mad1–Mad2 core complex and blocks the recruitment and activation of cytosolic O-Mad2.

Fig. 4. Mutual regulation between APC/C^{Cdc20} and the spindle checkpoint. APC/C targets spindle checkpoint proteins, including Bub1 and Mps1, for ubiquitination and degradation, and indirectly regulates the kinetochore-dependent production of MCC. When bound to APC/C, Cdc20 as a subunit of MCC undergoes autoubiquitination, which is stimulated by Ubch10 and p31^{comet} and removed by Usp44. Paradoxically, Cdc20 autoubiquitination has been proposed to regulate both checkpoint activation and inactivation.