

## Perspective

# Bub1 Multitasking in Mitosis

Hongtao Yu\*

Zhanyun Tang

Department of Pharmacology; The University of Texas Southwestern Medical Center; Dallas, Texas USA

\*Correspondence to: Hongtao Yu; Department of Pharmacology; the University of Texas Southwestern Medical Center; 5323 Harry Hines Boulevard; Dallas, Texas 75390 USA; Tel.: 214.648.9697; Fax: 214.648.2971; Email: hongtao.yu@utsouthwestern.edu

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mitosis, sister chromatid separation, sister chromatid cohesion, spindle checkpoint, Bub1, Cdc20, anaphase-promoting complex, cyclosome, cohesin, shugoshin, MEI-S332

### ABSTRACT

Accurate partition of duplicated genetic material to the daughter cells during mitosis relies on the maintenance of the physical linkage (cohesion) between sister chromatids until their bipolar attachment to the mitotic spindle. In response to a single straying chromatid within a cell, a surveillance mechanism called the spindle checkpoint blocks the ubiquitin ligase activity of the anaphase-promoting complex or cyclosome (APC/C), stabilizes securin (an APC/C substrate and an inhibitor of separase), and delays the activation of separase. This in turn prevents cleavage of cohesin by separase, preserves sister chromatid cohesion, and delays the onset of anaphase. The protein kinase, Bub1, is a key component of the spindle checkpoint. Bub1 has an upstream function in regulating the kinetochore localization of Mad2 and other downstream checkpoint components. In addition, recent biochemical studies have shown that Bub1 directly phosphorylates the APC/C activator, Cdc20, and inhibits APC/C. Finally, Bub1 has a noncheckpoint function at the kinetochores and preserves centromeric cohesion through the MEI-S332/shugoshin family of proteins. Therefore, Bub1 performs multiple tasks in mitosis that ensure the proper inheritance of chromosomes.

### INTRODUCTION

During the mitotic cell cycle, each chromosome is duplicated precisely once through semi-conservative DNA replication. The original and the duplicated copies of each chromosome (sister chromatids) remain associated with each other through sister chromatid cohesion, which is mediated by the cohesin protein complex.<sup>1,2</sup> In mitosis, the two opposing kinetochores (protein complexes assembled at centromeres) of each sister chromatid pair are captured by microtubules emanating from the two opposite spindle poles (bi-orientation).<sup>1</sup> After all sister chromatids have achieved bi-orientation, the anaphase-promoting complex or cyclosome (APC/C) tags securin with poly-ubiquitin chains, leading to its degradation by the proteasome.<sup>3,4</sup> Degradation of securin activates a protease called separase, which then cleaves the Scc1 subunit of cohesin, resulting in the loss of sister chromatid cohesion and the onset of sister chromatid separation.<sup>1</sup> The two sets of separated sister chromatids are then pulled toward opposite poles of the mitotic cell through their attachment to spindle microtubules. This elegant mechanism allows the perfect sorting of the sister chromatids into the two daughter cells. In the end, each daughter cell inherits an identical set of chromosomes.

Because capture of sister chromatids by microtubules occurs in a stochastic fashion, mechanisms must exist to prevent the precocious separation of sister chromatids that have already achieved microtubule-attachment to ensure the synchrony of chromosome segregation.<sup>5</sup> The spindle checkpoint is one such mechanism.<sup>6,7</sup> An elegant experiment in mammalian cells has demonstrated that a single unattached kinetochore is sufficient to activate the spindle checkpoint and impose a delay in the onset of anaphase.<sup>8</sup> The molecular components of the spindle checkpoint include, among other proteins, Mad1, Mad2, Mad3/BubR1, Bub1, Bub3, Mps1 and Aurora B.<sup>6,7,9</sup> In response to unattached kinetochores, these proteins collaborate to inhibit the ubiquitin ligase activity of APC/C, the main target of the spindle checkpoint, thereby stabilizing securin and delaying sister chromatid separation.<sup>7,9</sup> Among these checkpoint proteins, Mad3/BubR1 and Mad2 are capable of blocking the activity of APC/C through their direct binding to Cdc20, an activator of APC/C (Fig. 1).<sup>7,9</sup> Mad2 and Mad3/BubR1 are therefore downstream components of the checkpoint. In this article, we highlight the recent progress in our understanding of the roles of Bub1 in the spindle checkpoint and in preserving sister chromatid cohesion at or near the centromeres.

## FUNCTIONS OF BUB1 IN THE SPINDLE CHECKPOINT

Bub1 is a serine/threonine protein kinase. It was first isolated by Hoyt and coworkers in a screen searching for budding yeast mutants that were sensitive to the spindle poison, benomyl.<sup>10</sup> In budding yeast, hypomorphic alleles of Bub1 cause mitotic arrest defects in the presence of gross spindle damage.<sup>10</sup> Moreover, overexpression of a dominant mutant of Bub1 (Bub1-5) causes a Mad2-dependent mitotic delay that requires the kinase activity of Bub1-5.<sup>11</sup> Mad1 becomes hyperphosphorylated in Bub1-5-overexpressing cells.<sup>11</sup> In vertebrates, Bub1 localizes to kinetochores in mitosis and is required for the proper kinetochore localization of Mad1 and Mad2.<sup>12-14</sup> Interestingly, a kinase-inactive mutant of Bub1 is fully capable of recruiting Mad1 and Mad2 to the kinetochores in *Xenopus* egg extracts.<sup>12</sup> These and other findings indicate that Bub1 has an upstream role in the spindle checkpoint, possibly through phosphorylating down-stream checkpoint components and through recruiting Mad1 and Mad2 to kinetochores in a manner independent of its kinase activity (Fig. 1).

Recently, we have shown that mammalian Bub1 directly phosphorylates Cdc20.<sup>15</sup> Phosphorylation of Cdc20 by Bub1 inhibits the activity of APC/C in vitro. The same set of six Ser/Thr residues on Cdc20 is phosphorylated in mitotic HeLa cells and by recombinant purified Bub1 in vitro.<sup>15</sup> A Cdc20 mutant with these six residues mutated to Ala (Cdc20<sup>BPM</sup>) is refractory to Bub1 inhibition in vitro. Ectopic expression Cdc20<sup>BPM</sup> in HeLa cells impairs the function of the spindle checkpoint.<sup>15</sup> Because Cdc20<sup>BPM</sup> is still inhibited by Mad2 or BubR1 in vitro, the checkpoint defect of Cdc20<sup>BPM</sup>-expressing cells is likely due to the lack of inhibition by Bub1. Therefore, Cdc20 is a key substrate of Bub1 in the spindle checkpoint. It is unclear how phosphorylation of Cdc20 by Bub1 inhibits APC/C. These phosphorylation events on Cdc20 might prevent its binding to APC/C in a manner analogous to Cdk-mediated phosphorylation of Cdh1, a Cdc20-related APC/C activator.<sup>16-18</sup>

Rey-Hui Chen and coworkers have shown that a kinase-inactive mutant of Bub1 is capable of maintaining the mitotic arrest in *Xenopus* egg extracts supplemented with high concentrations of sperm nuclei and nocodazole.<sup>12</sup> On the other hand, the chromosome-bound Bub1 is hyperphosphorylated in checkpoint-active extracts, suggesting that the kinase activity of Bub1 is stimulated upon binding to chromosomes (Fig. 1).<sup>19</sup> Moreover, the kinase activity of Bub1 is required for the mitotic arrest in *Xenopus* extracts that contain low concentrations of sperm nuclei or nocodazole.<sup>19</sup> These findings are consistent with the notion that Bub1 is required for the ability of the spindle checkpoint to sense small numbers of unattached kinetochores. We have shown that Bub1 isolated from nocodazole-treated HeLa cells is more active in phosphorylating Cdc20.<sup>15</sup> Thus, phosphorylation of Cdc20 by Bub1 may partly account for the exquisite sensitivity of the spindle checkpoint (Fig. 1). In the future, it is important to determine the relative contribution of each APC/C-inhibitory checkpoint mechanism to the maintenance of chromosomal stability during the normal cell cycle (i.e., in the absence of

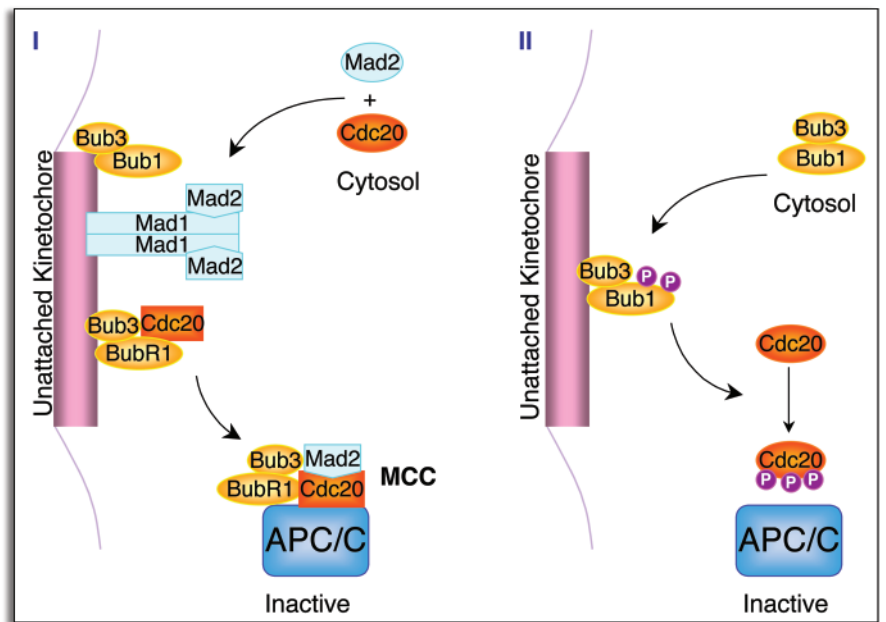


Figure 1. Functions of Bub1 in the spindle checkpoint. In the first function (I), Bub1 is required for the recruitment of Mad1 and Mad2 to kinetochores. Mad1 triggers a conformational change of Mad2, which facilitates its binding to Cdc20.<sup>6,38,40</sup> This leads to the efficient formation of the mitotic checkpoint complex (MCC) containing BubR1-Bub3-Mad2-Cdc20, which then inhibits APC/C.<sup>7</sup> Direct evidence for the formation of MCC at the kinetochores is lacking. In the second function (II), the kinase activity of Bub1 is stimulated at the kinetochores. The activated Bub1 phosphorylates Cdc20 and inhibits APC/C.

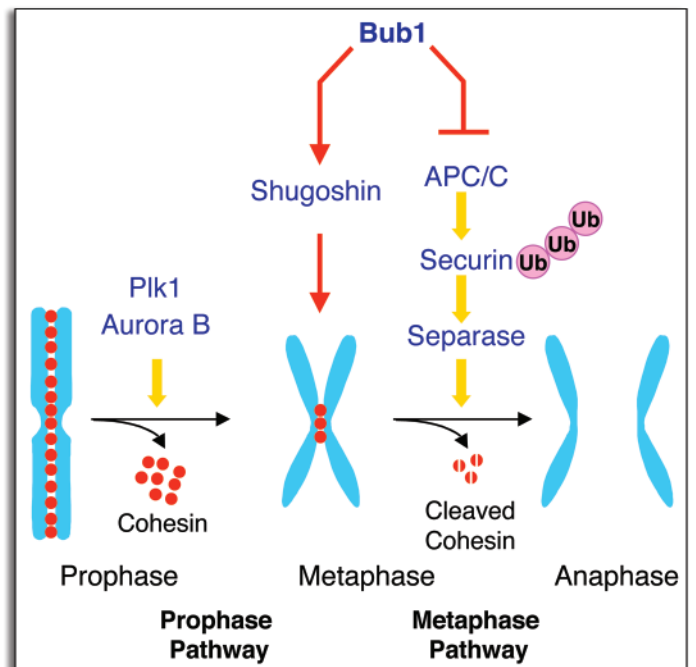


Figure 2. The noncheckpoint kinetochore function of Bub1. Sister chromatid cohesion is maintained by the cohesin protein complex. Cohesin is removed from sister chromatids by two distinct mechanisms in vertebrate cells: (1) Plk1/Aurora B-dependent phosphorylation of arm cohesin; (2) separate-mediated cleavage of centromeric cohesin. In addition to its spindle checkpoint functions in APC/C-inhibition shown in Figure 1, Bub1 is required for the stability and centromeric localization of shugoshin, which in turn is required for the protection of centromeric cohesin through yet unknown mechanisms.

gross spindle damage). This will require the development of additional, more precise assays for chromosome mis-segregation events, instead of the commonly used assays that examine the lack of mitotic arrest and the consequent polyploidy in the presence of spindle poisons.

## FUNCTIONS OF BUB1 IN THE PROTECTION OF CENTROMERIC COHESION

Loss of sister chromatid cohesion triggers the onset of anaphase. In vertebrate cells, removal of cohesin occurs in two steps (Fig. 2).<sup>20</sup> At prophase, most of cohesin along the chromosome arms is removed through Plk1/Aurora B-dependent phosphorylation of cohesin.<sup>21,22</sup> At metaphase, the residual centromeric pool of cohesin is cleaved by separase to allow sister chromatid separation.<sup>23</sup> An interesting puzzle is how the centromeric cohesin is shielded from the actions of Plk1/Aurora B in prophase. In addition, cohesin at the centromeres of sister chromatids is protected from the actions of separase during meiosis I.<sup>24</sup> The MEI-S332/shugoshin family of proteins is critical for the protection of centromeric cohesion.<sup>25</sup> Each shugoshin protein contains an N-terminal coiled-coil and a C-terminal basic domain, both of which are required for its localization to centromeres.<sup>26</sup> Loss of shugoshin causes chromosome mis-segregation during mitosis and meiosis I in organisms from yeast to man.<sup>27-32</sup> These data indicate that shugoshin is involved in the protection of centromeric cohesin. In addition, the vertebrate Sgo1 is an APC/C substrate and regulates kinetochore-microtubule attachment.<sup>32</sup>

Genetic studies in yeast have revealed a role of Bub1 at the kinetochores that is distinct from its role in the Mad2-dependent spindle checkpoint.<sup>33,34</sup> Loss of Bub1 abolishes the localization of shugoshin at or near the centromeres in fission yeast, suggesting that shugoshin is a critical target of Bub1 at the kinetochores.<sup>29</sup> This kinetochore function of Bub1 is conserved in mammalian cells.<sup>35</sup> Depletion of Bub1 or Sgo1 by RNAi causes massive chromosome mis-segregation in HeLa cells.<sup>35</sup> In Bub1-RNAi cells, the level of Sgo1 protein is lower, and the residual amount of Sgo1 fails to localize to centromeres.<sup>35</sup> This suggests that Bub1 regulates the stability and centromeric localization of Sgo1 in human cells. It remains to be established whether shugoshin is a direct substrate of Bub1. It is also unclear how shugoshin protects centromeric cohesin from Plk1/Aurora B and/or separase.

Interestingly, despite having separated sister chromatids, Sgo1-RNAi cells undergo a Mad2/Aurora B-dependent mitotic arrest, suggesting that the sister chromatid separation in these cells does not require full activation of separase.<sup>32,35</sup> Indeed, RNAi-mediated depletion of separase does not prevent the massive chromosome mis-segregation in Sgo1-RNAi cells.<sup>35</sup> Thus, the aberrant sister chromatid separation in Sgo1-RNAi cells might be triggered by Plk1/Aurora B. Alternatively, Sgo1 might play a direct role in the establishment/maintenance of sister chromatid cohesion. Unfortunately, due to the multiple roles of Plk1 and Aurora B in mitosis, experiments involving codepletion of Plk1 and Aurora B from Sgo1-RNAi cells have not been informative (Tang Z, Yu H, unpublished results).

Paradoxically, Bub1-RNAi cells accumulate in mitosis in the absence of spindle damage. This accumulation is dependent on Mad2 and Aurora B.<sup>35</sup> However, there is a fundamental difference between the cell-cycle phenotypes of Sgo1- and Bub1-RNAi cells. In the presence of nocodazole, the Sgo1-RNAi cells undergo efficient mitotic arrest whereas many Bub1-RNAi cells escape mitosis.<sup>15</sup>

These data suggest that Bub1-RNAi cells only exhibit a transient delay in mitosis whereas Sgo1-RNAi cells undergo a robust mitotic arrest. This is consistent with the aforementioned roles of Bub1 in the spindle checkpoint. If Bub1 does not play a role in the spindle checkpoint, Bub1 inactivation is expected to cause a mitotic-arrest phenotype, as robust as that of the Sgo1-RNAi cells. A similar situation exists for Aurora B in budding yeast. On the one hand, Aurora B is required for the bi-orientation of sister chromatids.<sup>36</sup> On the other hand, inactivation of Aurora B and the consequent improper kinetochore-microtubule attachment do not activate the spindle checkpoint.<sup>37</sup> One simple explanation is that Aurora B is also a component of the spindle checkpoint, and is required for sensing the very defect in kinetochore-microtubule attachment that is caused by its own inactivation.<sup>37</sup> Strategies that allow the separation of the multiple functions of Bub1 and Aurora B will be invaluable for future studies on the roles of these proteins in mitosis.

## CONCLUSION

Bub1 performs multiple, distinct functions in mitosis. As a critical component of the spindle checkpoint, Bub1 has an upstream function in recruiting Mad1 and Mad2 to the kinetochores and a downstream function in phosphorylating Cdc20 and inhibiting APC/C. Independently of its checkpoint function, Bub1 is also required for the stability and kinetochore localization of shugoshin and maintains sister chromatid cohesion at centromeres.

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