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MINIREVIEW

Bub1 and BubR1: at the Interface between Chromosome Attachment and the Spindle Checkpoint▽

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The spindle checkpoint ensures genome fidelity by temporarily halting chromosome segregation and the ensuing mitotic exit until the last kinetochore is productively attached to the mitotic spindle. At the interface between proper chromosome attachment and the metaphase-to-anaphase transition are the mammalian spindle checkpoint kinases. Compelling evidence indicates that the checkpoint kinases Bub1 and BubR1 have the added task of regulating kinetochore-microtubule attachments. However, the debate on the requirement of kinase activity is in full swing. This minireview summarizes recent advances in our understanding of the core spindle checkpoint kinases Bub1 and BubR1 and considers evidence that supports and opposes the role of kinase activity in regulating their functions during mitosis.

Maintenance of genome stability is necessary to ensure the continued survival of progeny throughout multiple rounds of division. In mitosis, the shortest but most visually striking phase of the cell cycle, accurate distribution of chromosomes to the nascent progeny requires proper attachment of the duplicated chromosome (sister chromatid pair) to microtubules emanating from opposite poles of the mitotic spindle and their subsequent alignment to the spindle equator. The site of microtubule attachment is the kinetochore, a conserved, proteinaceous network that assembles onto chromosomes upon mitotic entry (45, 73, 82). In addition to its structural role, the enrichment of kinases, phosphatases, and other modifying enzymes to its various substructures support its function as a signaling hub during mitosis. Microtubule capture by kinetochores is a highly dynamic and stochastic process involving numerous protein complexes and a multitude of weak microtubule binding sites (11, 58, 90). Not surprisingly, errors in attachment do occur in early mitosis; these include syntelic attachments, which involve microtubules from a single pole binding both sister chromatids, and merotelic attachments, which occur when a kinetochore is attached to microtubules emanating from both poles. Most misattachments, however, are sensed and corrected, given sufficient time. The spindle checkpoint (also known as the spindle assembly checkpoint and the mitotic checkpoint) is a conserved surveillance mechanism that provides this extra time when necessary. Importantly, this checkpoint does not permanently arrest cells in mitosis. Rather, it delays mitotic progression until all kinetochores are attached (62, 63). Whether microtubule attachment itself or the tension generated at kinetochores as a result of this attachment satisfies the spindle checkpoint is vigorously debated and is the subject of a number of excellent recent reviews (57, 65, 72). The duration of a spindle checkpoint-mediated arrest is highly variable and appears to be cell type and organism dependent (24, 77). Moreover, the activity of certain checkpoint kinases (see below) may modulate the length of a checkpoint-mediated arrest. Cells that do not satisfy the checkpoint often die or exit mitosis into the next G_1 as single tetraploid cells via poorly understood "slippage" or "adaptation" pathways (77). The importance of accurate and stable microtubule attachments to the regulation of checkpoint signaling is underscored by increasing evidence that points to an active role for the spindle checkpoint kinases during the establishment of attachments.

SPINDLE CHECKPOINT SIGNALING

The core components of the spindle checkpoint were originally identified in the budding yeast *Saccharomyces cerevisiae* and include the budding uninhibited by benzimidazole (Bub) proteins Bub1 and Bub3 (29, 49, 80) and the mitotic-arrest deficient (Mad) proteins Mad1, Mad2, and Mad3 (BubR1 in higher eukaryotes). Subsequently, the dual-specificity kinase monopolar spindle 1 (Mps1), which is required for spindle pole body (SPB) duplication in yeast, was also shown to be essential for spindle checkpoint function (26, 100). For most of these proteins, checkpoint function is conserved from yeast to humans as well as in plants (7). Checkpoint signaling, however, may be a more elaborate process in metazoans. In budding yeast for example, full attachment is achieved by the binding of a single microtubule to each kinetochore, whereas it is estimated that 25 to 30 microtubules attach per kinetochore in mammals (44, 78).

The only known target of the spindle checkpoint is Cdc20, a substrate binding subunit of the anaphase-promoting complex/cyclosome (APC/C) (71). The APC/C is a large, multisubunit E3 ubiquitin ligase that targets two key proteins during mitosis, cyclin B and securin. Cyclin B is an obligatory activating part-

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ner of the major mitotic kinase Cdk1, and its degradation allows for rapid Cdk1 inactivation and the ensuing spindle disassembly and mitotic exit. Loss of securin releases active separase, which cleaves the cohesin rings holding sister centromeres together (45, 62, 63). How the spindle checkpoint functions to attenuate APC/C^{Cdc20} is an intense field of research. In particular, the role of posttranslational modifications of both the APC/C^{Cdc20} and core checkpoint components remains controversial. Considerable evidence points to Mad2 and BubR1 as the ultimate arbitrators of the "wait anaphase" signal. Elegant structural and biochemical studies demonstrated that Mad2 exists in two distinct structural conformations, open O-Mad2 and closed C-Mad2. Mad1 at the kinetochore binds stably to C-Mad2, and soluble O-Mad2 dimerization with kinetochore-bound C-Mad2 catalyzes the release of C-Mad2, which is capable of Cdc20 binding and inhibition (52, 56) (Fig. 1A). More recently, the function of Mad3/BubR1 in Cdc20 inhibition and checkpoint signaling has garnered increasing attention. Mad3/BubR1 orthologues contain two KEN (Lys-Glu-Asn) motifs. Although these motifs usually mediate APC-substrate recognition and ubiquitination, two key studies showed that in *S. cerevisiae* Mad3, these motifs are essential for checkpoint function (5, 40) (Fig. 1B). These observations have been verified in fission yeast (83), flies (74), rodents (53), and human cells (19). While the N-terminal KEN box of Mad3/BubR1 orthologues binds directly to Cdc20 and may be involved in Cdc20 degradation, the C-terminal motif does not (19, 40, 83); rather, it has been proposed that this motif mediates an interaction between Mad3/BubR1 and the core APC/C (19), although this remains to be demonstrated. Remarkably, the molecular mechanisms of the spindle checkpoint may be conserved across kingdoms, as both KEN box motifs are present in the recently identified *Arabidopsis thaliana* Mad3 (7).

Both Mad2 and Mad3/BubR1 are required for the checkpoint *in vivo*, suggesting that they function cooperatively. The prevalent model of checkpoint signal transduction suggests that Mad3/BubR1 binding to Cdc20 may require prior priming of Cdc20 by Mad2 (14, 40, 68, 85), a process that is greatly expedited at unattached kinetochores that generate C-Mad2 (46) (Fig. 1C). Mad2 may be subsequently released from the inhibited APC/C^{Cdc20} complex, leaving bound Mad3/BubR1 to inhibit securin and cyclin B polyubiquitination (68). How Mad2 stimulates Mad3/BubR1 binding to Cdc20 is clearly a question for future studies. Curiously, some evidence suggests that Mad2 and BubR1 Cdc20-inhibitory complexes may form and function to some extent independently. Mad2 and BubR1 can individually bind and inhibit APC/C^{Cdc20} *in vitro* (22, 32, 91), and reconstitution of checkpoint signaling *in vitro* demonstrated that Mad2 inhibition of APC/C^{Cdc20} is kinetochore-dependent, whereas BubR1 binding and inhibition of Cdc20 do not require kinetochores (46). Moreover, double small interfering RNA (siRNA)-mediated depletion of Mad2 and BubR1 from mammalian cells appears to accelerate progression through mitosis more than single depletions (60), which would not be expected if their functions were entirely interdependent. Clearly, a full understanding of APC/C^{Cdc20}-inhibitory mechanisms remains to be realized.

THE SPINDLE CHECKPOINT KINASES

The response of the spindle checkpoint to the state of kinetochore-microtubule attachment is exquisitely fine-tuned such that a single unattached kinetochore generates sufficient "wait anaphase" signal to arrest a cell in mitosis (76). In addition, the establishment of productive kinetochore-microtubule interactions, the event monitored by the spindle checkpoint, is a dynamic process during which microtubules are rapidly captured and released to allow correct attachment formation. The expeditious and precise nature of these events implies that signals must be rapidly and efficiently turned on and off. Classical experiments have suggested that biophysical changes caused by microtubule attachment and tension generation translate into a biochemical signal reflected by the phosphorylation state of a kinetochore epitope recognized by the 3F3/2 monoclonal antibody (8, 66, 67). These observations laid the foundation for the hypothesis that kinase activity is required to maintain an active checkpoint and must be inhibited or counteracted for anaphase to proceed (8). This model is particularly attractive in light of the number of kinases involved in spindle checkpoint signaling either directly through APC/C^{Cdc20} binding and inhibition or indirectly through modulating microtubule attachments (Table 1). Here I discuss the structurally related kinases Bub1 and BubR1, with emphasis on the implications of catalytic activity for the spindle checkpoint and chromosome alignment.

Bub1. (i) Scaffolding functions of Bub1. Bub1 is one of the first checkpoint components to dock at the nascent kinetochore in early prophase (33) and is a true checkpoint protein; cells in which Bub1 function is ablated do not arrest in response to microtubule poisons (1a, 2, 29, 86, 96). Bub1 recruitment to the kinetochore occurs through a direct interaction between its N-terminal tetratricopeptide repeat (TPR) domain and blinkin (also known as hKNL1, AF15q14, D40, and CASC5), a member of the conserved KMN (KNL1/Mis12 complex/Ndc80 complex) network of kinetochore proteins (10, 11). Studies of fluorescence recovery after photobleaching in both yeast and human cells have indicated that Bub1 is a stable component of the kinetochore and may act as a scaffold for coordinating checkpoint signaling (28, 79, 84). Indeed, artificially tethering Bub1 to telomeres in yeast is sufficient for the ectopic recruitment of downstream checkpoint components in a kinase-independent manner (79). In this capacity, Bub1 determines the kinetochore recruitment of a number of targets, including centromere proteins E and F (Cenp-E and Cenp-F, respectively), Bub3, Mad3/BubR1, the mitotic centromere-associated kinesin (MCAK), Mad1, and Mad2 (3, 30, 35, 42, 61, 86, 98). In particular, a conserved region (CDI) between amino acids 458 and 467 of hBub1 is required for Mad1, Mad2, and BubR1 recruitment and consequently for checkpoint function (43). Moreover, Bub1 also regulates the targeting of the MEI-S332/shugoshin (Sgo) proteins to the centromere during both meiosis and mitosis (41, 93, 94), although this may be kinase dependent (69). Interestingly, recent observations in mammalian cells have suggested that cytoplasmic Bub1 is at least partially functional (43).

(ii) Bub1 kinase activity and the spindle checkpoint. In budding yeast, expression of a stable truncated mutant of Bub1 entirely lacking the kinase domain supports a functional check-

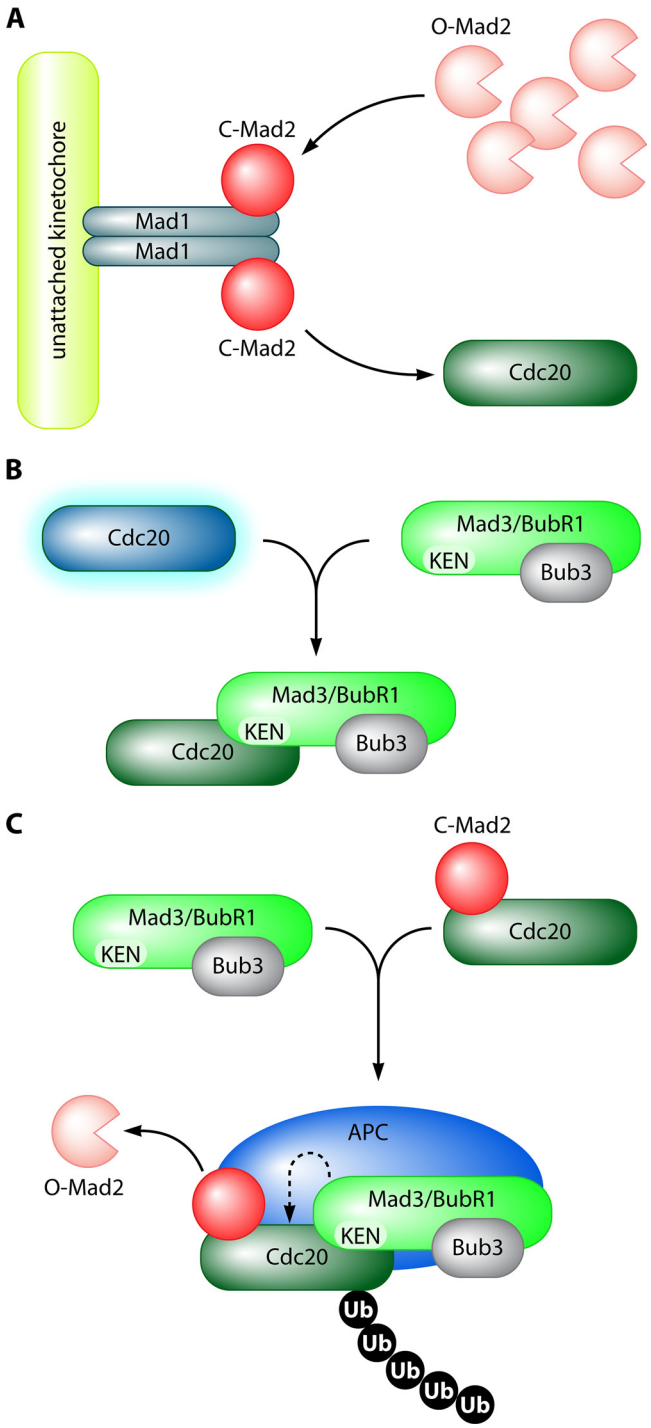

FIG. 1. The "wait anaphase" signal is generated at improperly attached kinetochores. (A) Mad2 exists in two major conformations, open (O-Mad2, light red indented circles), which is mainly a free, cytoplasmic form, and closed (C-Mad2, bright red circles), which is either Mad1 or Cdc20 bound. Kinetochore-bound Mad1 dimers associate with Mad2 in the closed form at unattached kinetochores. Dimerization between O-Mad2 and C-Mad2 results in the release of a C-Mad2 molecule capable of binding to and inhibiting Cdc20; (inhibited Cdc20 is shown in green). The pathway for APC/C^{Cdc20} inhibition is kinetochore dependent. (B) Mad3/BubR1-Bub3 can bind to and inhibit active Cdc20 (shown in blue) independent of Mad2 and kinetochores. Through its N-terminal KEN motif, BubR1 can bind directly to and inhibit Cdc20. It is not clear if Cdc20 changes structural conformation upon Mad2 or Mad3/BubR1 binding. (C) Both Mad2 and Mad3/BubR1 are required for checkpoint function *in vivo* and may function cooperatively to mediate APC/C^{Cdc20} inhibition. Prior binding to Mad2 may prime Cdc20 for the interaction with the Mad3/BubR1-Bub3 complex. Mad3/BubR1 inhibits APC/C^{Cdc20} activity by acting as a pseudosubstrate and/or by mediating Cdc20 ubiquitination and degradation, as denoted by the dashed arrow. Mad2 may dissociate from the inhibited APC/C^{Cdc20} complex once it is formed, being released into the cytoplasm again as free O-Mad2.

point (23, 98). In contrast, in fission yeast, while some studies indicated a robust checkpoint response in cells lacking Bub1 kinase activity (37, 79), others have suggested that kinase function is required but not sufficient for checkpoint signaling (103). Similarly, a kinase-inactive Bub1 in *Xenopus* egg extracts supports the checkpoint, albeit at high concentrations of the microtubule-depolymerizing drug nocodazole, and this effect was attenuated at low concentrations (86). In mammals, the general consensus is that Bub1 kinase activity is expendable for mounting a spindle checkpoint response. It may, however, play a subtle role in fine-tuning the arrest. Recent studies have addressed the function of Bub1 kinase using inactivation-complementation approaches in mammals. Using a floxed allele, Bub1 was specifically inactivated in mouse oocytes, resulting in premature APC/C activation in meiosis I, indicating that a Bub1-dependent checkpoint response can be generated in oocytes (59). The delay in APC/C activation normally imposed by endogenous Bub1 could be rescued by both active and inactive Bub1 kinase, although the latter was somewhat less efficient. In murine somatic cells however, Bub1 depletion was rescued to the same extent by both active and inactive Bub1 kinase, indicating that in this context Bub1 catalytic activity is not essential for the checkpoint signaling (69, 70). Similarly, In hTERT-RPE1 cells Bub1 kinase activity appeared to be dispensable for the checkpoint, whereas somewhat surprisingly, the same study indicated that checkpoint activity was more efficient in the presence of active Bub1 in HeLa cells (43). Therefore, while the kinase activity of Bub1 does not regulate the checkpoint in a switch-like manner, it may modulate the strength of the checkpoint signal and delay APC/C^{Cdc20} activation and anaphase onset in certain cell lines or developmental contexts.

What are the potential targets of Bub1 kinase activity relevant to the spindle checkpoint? *In vitro*, human Bub1 can inhibit the APC/C when bound to Cdc20; in contrast, a catalytically inactive mutant cannot. This inhibition might be direct, as Bub1 can phosphorylate Cdc20 *in vitro* (92). Although *in vivo* phosphorylation sites identified on Cdc20 have been attributed to Bub1, this remains to be formally demonstrated. Nevertheless, a nonphosphorylatable Cdc20 mutant does not support mitotic arrest in response to nocodazole or taxol treatment to the same extent as wild-type Cdc20, arguing that phosphorylation may be critical for a checkpoint-mediated arrest. Importantly, Cdc20 has been reported to be a mitogen-activated protein kinase (MAPK) substrate in *Xenopus*, and mutation of the MAPK phosphorylation sites also weakens the checkpoint response (13). Whether Cdc20 is also a MAPK target in mammals is not known.

TABLE 1. Mitotic kinases required for spindle assembly, congression, and the spindle checkpoint[a]

Family	Mammalian mitosis-specific member	Mitotic functions	Reference(s)
Cdk	Cdk1 (together with cyclins A and B)	Mitotic entry, nuclear envelope breakdown, chromosome condensation, bipolar spindle assembly, microtubule attachment, APC/C regulation, spindle checkpoint regulation	20a, 67a
Polo kinase	Plk1	Mitotic entry, centrosome maturation, cohesion removal, bipolar spindle assembly, microtubule attachment stability, cytokinesis	1, 71a
Aurora kinase	Aurora A	Mitotic entry, Plk1 activation in late G_2, centrosomes maturation, centrosome-dependent and -independent bipolar spindle formation	8a, 47, 48b
	Aurora B	Catalytic subunit of the chromosomal passenger complex, spindle assembly, correction of tensionless microtubule attachments, sister chromatid and centromeric cohesion removal, cytokinesis and furrow ingression, kinase activity may regulate a prolonged spindle checkpoint-mediated arrest	
	Aurora C	Meiotic functions, somatic role unclear	
NIMA kinase	NEK2A	Mitotic and meiotic spindle assembly, centrosome splitting, microtubule attachment stability	68a
	NEK6	Mitotic progression	68b, 103a
Mitotic checkpoint kinase	Bub1	Regulation of end-on microtubule attachments at kinetochores, regulates the spindle checkpoint through recruitment of Mad1, Mad2, and Mad3/BubR1	2a, 96a, 96b
	BubR1	Regulation of microtubule attachment stability, core checkpoint component through APC/C^{Cdc20} inhibition	2a
	hMps1/TTK (Hs), Esk (Mm)	Correction of improper microtubule attachments, control of the spindle checkpoint through kinetochore recruitment of Mad1 and Mad2, kinetochore recruitment of the Rod-Zwilch-ZW10 complex	27a, 34a, 48a, 52a, 82a
MAPK	MAPK1/2, p38	Phosphorylation of Cdc20, Mps1, and Bub1; mitotic progression; kinetochore-microtubule attachment	13, 13a, 105
Haspin	Haspin	Histone H3 phosphorylation, chromosome congression	27b
Pre-mRNA processing	PRP4	Kinetochore recruitment of Mps1, Mad1, and Mad2	61a
ILK	ILK	Spindle assembly	23a

[a] This table is not meant to be exhaustive, and kinases not involved in the mitotic checkpoint or spindle assembly and stability are not included.

(iii) Bub1 kinase function and chromosome congression. In all tested organisms, impairment of Bub1 function causes congression defects. In both budding and fission yeasts, Bub1 deletion results in severe chromosome missegregation at levels that are elevated compared to those observed after depletion of other checkpoint proteins such as Mad1, Mad2, and Mad3 (23, 97, 98). In human cells, knockdown of Bub1 by RNA interference (RNAi) also causes errors in chromosome alignment resulting from the accumulation of lateral attachments and the consequent delay in formation of stable end-on attachments (17, 25, 50, 61).

There is compelling evidence that the kinase activity of Bub1 contributes to its role in chromosome congression and alignment. Fission and budding yeasts expressing inactive Bub1 are remarkably sensitive to microtubule drugs and display defects in biorientation and chromosome missegregation (23, 97, 98). In an isogenic siRNA complementation system in human cells, Bub1 kinase activity was necessary for precise chromosome alignment (43), in agreement with observations in yeast. However, in murine cells, the equivalent mutants effectively restored proper alignment, suggesting that the kinase activity of Bub1 may not be required for biorientation in this context (69). The surprising discrepancy between the murine and human Bub1 findings may be due to inefficient levels of exogenous protein expression or may reflect the inherent variations in alignment efficiency between organisms and cells types. The question remains as to the targets of Bub1 that direct end-on attachment, although the Sgo proteins are attractive candidates (see below) (81).

(iv) Bub1 activity and Sgo1. Following DNA replication, sister chromatid pairs are held together in part due to the cohesin protein complex, which is thought to form a ring around the newly replicated DNA (64, 87). At prophase, most of the cohesion is removed from chromosome arms as a consequence of phosphorylation by Plk1 and Aurora B (51, 89). The residual centromeric pool is protected from phosphorylation by Sgo. Whereas budding yeast and flies have only one Sgo protein, fission yeast, plants, frogs, and mammals have two Sgo-like proteins, the mitosis-specific Sgo1 and Sgo2, which is expressed during both meiosis and mitosis (99). Recruitment and maintenance of Sgo proteins at the centromere are complex. In prometaphase Bub1 directs centromeric localization of Sgo proteins, and attenuation of either Sgo or Bub1 results in loss of sister chromatid cohesion and chromosome missegregation (41, 94). Centromere recruitment of Sgo is dependent on Bub1 kinase activity in yeast during mitosis (23) and meiosis (103). Consistently, in frogs and in human cells, Bub1 kinase activity is necessary for Sgo centromere localization (3, 43, 69). Bub1 also directs the PP2A phosphatase to centromeres, where it maintains Sgo protection by counteracting Plk1-mediated phosphorylation (41, 75, 93). Phosphorylation of histone H2A may also facilitate Sgo localization. Bub1 phosphorylates the conserved S121 of fission yeast histone H2A *in vitro* and *in vivo*. In yeast and human cells, mutation of this residue

phenocopies inactive Bub1, resulting in disruption of centromeric Sgo and microtubule attachments (37). In fission yeast and mouse embryonic fibroblasts (MEFs), Swi6/heterochromatin protein 1 (HP1α) has also been implicated in Sgo localization through a direct interaction between the chromo-shadow domain of Swi6/HP1 and Sgo1 (102). A potentially interesting twist comes from the observation that while Bub1 kinase activity appears to be necessary for Sgo recruitment to the centromere during mitosis, Sgo1's functions in late G_2 rather than prophase may confer its protective activity toward centromeric cohesion (69). Indeed, Bub1 may protect centromeric cohesion by regulating the checkpoint, rather than through direct regulation of Sgo1 function (70). This intriguing discovery implies that Bub1-mediated Sgo recruitment in mitosis may serve an entirely different function, perhaps regulation of kinetochore-microtubule attachments. In concordance with this idea, *Xenopus* Sgo was identified by virtue of its ability to bind and polymerize microtubules (81).

BubR1. BubR1 was initially identified by virtue of it its homology to Bub1 (6). It was shortly thereafter recognized as the mammalian orthologue of yeast Mad3, and its indispensable function in the checkpoint was confirmed in mammals (9, 95). BubR1 is found in higher eukaryotes, whereas Mad3, which clearly lacks a kinase domain, is expressed in yeasts and plants. This difference implies that BubR1 has acquired additional functions for which the kinase is required. Although the nature of these remains contentious, a role in both the spindle checkpoint and microtubule attachment stability has been proposed.

(i) BubR1 kinase activity and the spindle checkpoint. Initial studies in frogs indicated that a lack of BubR1 kinase activity does not interfere with Cdc20 binding or APC/C^{Cdc20} inhibition (21, 91). Indeed, BubR1 entirely lacking the kinase domain still supports the checkpoint (12, 14, 27, 53). These conclusions have been disputed by other *Xenopus* studies, however, which suggest that minimal BubR1 kinase function is indispensable for the checkpoint and that this activity is silenced as a result of spindle microtubule capture by Cenp-E (54, 55). A recent study reports an allosteric Cenp-E inhibitor (GSK923295) (101). Treatment of cells with this inhibitor mimicked the phenotype observed after Cenp-E depletion and antibody microinjection, characterized by an increase in the mitotic index and chromosomes lagging at the spindle poles. Importantly, this inhibitor locks Cenp-E in a microtubule-bound state; thus, in contrast to the situation in *Xenopus* extracts, binding of the Cenp-E motor domain to microtubules *per se* appears to be insufficient to satisfy the spindle checkpoint in human cells. Although the significance of the Cenp-E interaction with BubR1 remains unclear, the development of small-molecule inhibitors of both BubR1 and Cenp-E together with structural studies of BubR1–Cenp-E complexes will pave the way to answering some of the lingering questions.

The argument for a subtle BubR1 kinase function in the checkpoint has also been inferred from recent studies with mammalian cells. In MEFs both inactive BubR1 and the N-terminal Mad3-homologous region are capable of Cdc20 and APC/C binding and support growth and survival at the cellular level. However, the checkpoint response to a prolonged nocodazole challenge under these conditions is attenuated, suggesting that BubR1 kinase activity may contribute to the maintenance rather than the initiation of a checkpoint (27, 53). In keeping with this, flies expressing catalytically inactive BubR1 are viable and fertile and retain a functional checkpoint. Nevertheless, premature sister chromatid separation (PSCS), a sign of untimely mitotic exit and therefore checkpoint failure, is elevated in BubR1-KD flies (74). Taken together, these data support a model where BubR1 kinase activity is not essential during normal growth and development. The tight control over checkpoint function by the N-terminal Mad3 domain is sufficient to ensure viable progeny, at least during normal unperturbed mitoses. Kinase function may become important under conditions that prolong mitosis or when the checkpoint signal is weakened, as is expected when only a few kinetochores remain unattached, or under conditions that challenge attachment stability. Such a model leads to several important predictions. First, during a normal undisrupted mitosis, the BubR1 kinase domain and activity are not essential for the checkpoint. This is supported by the observations that mitotic timing in HeLa cells is not affected by BubR1 kinase activity, that cell viability does not require the kinase domain, and that BubR1-KD flies are viable and fertile (20, 53, 74). However, it will be important to verify these observations during development in knock-in mice, as the spindle checkpoint is not strictly essential in flies (4). Second, if the BubR1 kinase activity is indeed required for maintaining the checkpoint, it is reasonable to expect kinase targets to have checkpoint functionality. This has not yet been demonstrated for proposed substrates of BubR1 such as the microtubule plus-end protein adenomatous polyposis coli (36). Conversely, no genuine spindle checkpoint proteins have been shown to be definitively phosphorylated by BubR1. An important caveat is that the integrity of the BubR1 kinase domain is also critical for protein stability and may indirectly modulate the checkpoint through controlling BubR1 levels. Mutations in the kinase domain of BubR1 have been linked to the human cancer predisposition syndrome mosaic variegated aneuploidy, and cells from these patients display reduced BubR1 protein abundance and a defective checkpoint response to microtubule insult (88). It will be important to uncouple kinase activity from protein stability in order to definitively determine whether BubR1 kinase function is required for the checkpoint. Strikingly, we have also observed that mutations in the Bub1 kinase domain cause reduced Bub1 protein expression, suggesting that Bub1 protein stability may be regulated in a similar fashion (unpublished observations).

(ii) BubR1 kinase activity and chromosome alignment. A dual role for BubR1 in both checkpoint signaling and chromosome alignment was first recognized by Taylor and colleagues, who reported a reduction in metaphase (and increase in prometaphase) cells when transition to anaphase was blocked in BubR1-depleted cells, suggesting that BubR1 is indeed required for chromosome alignment (17). High-resolution microscopy subsequently demonstrated that the microtubule attachments were unstable in BubR1-depleted cells (48), and several lines of evidence suggest that proper alignment may depend on BubR1 kinase activity. When overexpressed, inactive BubR1 prolonged prometaphase in HeLa cells, a phenotype commonly attributed to lack of stable attachments (27). Similarly, neuroblasts from flies expressing catalytically dead BubR1 displayed a tendency toward a prolonged prometaphase characterized by slow congression and difficulty remain-

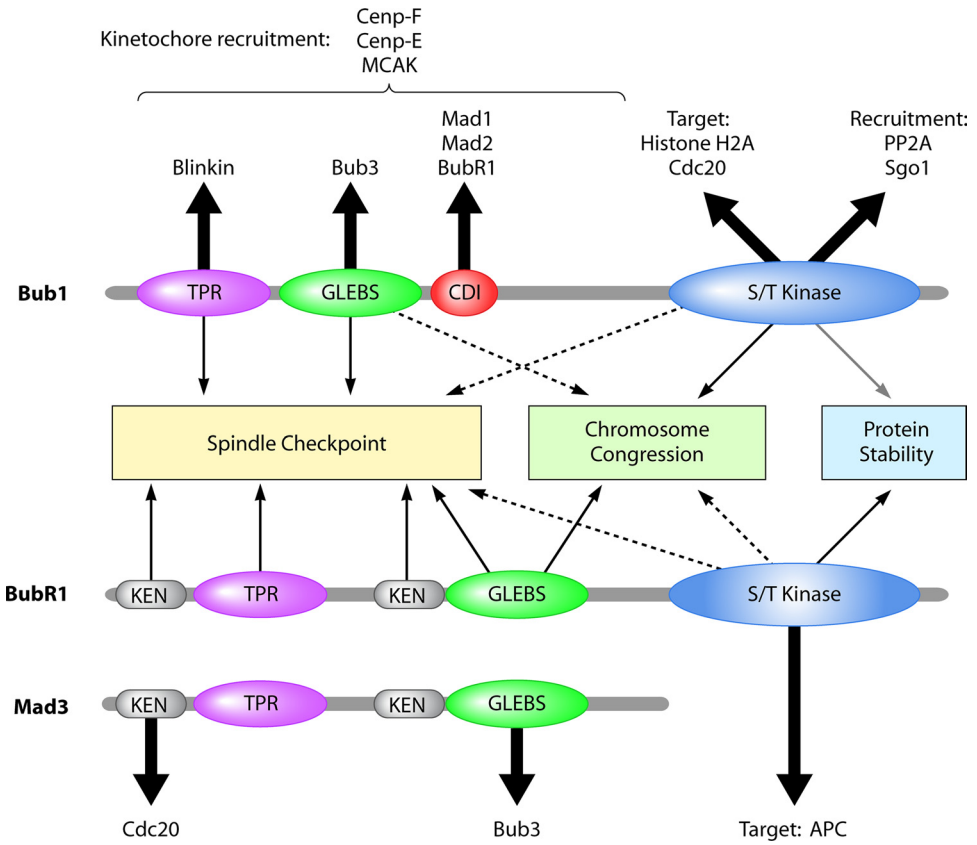

FIG. 2. Summary of the functions, interacting partners, and targets of the spindle checkpoint kinases Bub1 and BubR1. The domain architectures of Bub1, BubR1, and its orthologue Mad3 are illustrated, and the contribution of each motif to the mitotic checkpoint or to chromosome congression and biorientation is shown. A solid arrow indicates a clear requirement for the motif for a particular function across evolution, whereas a dashed arrow indicates that the contribution of the domain remains controversial. The gray arrow indicates our own unpublished results. Bold arrows indicate the interaction partners identified to date for each of the domains and interaction motifs highlighted in Bub1 and Mad3/BubR1. In the case of Cenp-F, Cenp-E, and MCAK, their kinetochore recruitment is Bub1 kinase independent.

ing aligned (74). In MEFs and HeLa cells, most inaccurate kinetochore-microtubule attachments are corrected in the absence of BubR1 kinase function, albeit inefficiently; however, BubR1 inactivation did not cause a mitotic delay (20, 31, 53). One possible interpretation of these results is that BubR1 kinase activity is required for the checkpoint (31). An alternative view is that timely anaphase onset may have been initiated, as the type of defects observed upon loss of BubR1 activity, such as merotelic attachments, are not detected by the spindle checkpoint.

Outer-kinetochore and microtubule plus-end binding proteins constitute attractive candidate substrates for BubR1 in its capacity to regulate attachment stability. Adenomatous polyposis coli and its binding partner EB1 are plus-end binding proteins that localize to kinetochores. Their depletion results in little or no delay in chromosome congression, but cells displayed lagging strands during anaphase, a phenotype strikingly similar to that caused by BubR1 kinase inactivation (18). As EB1- and adenomatous polyposis coli-depleted cells arrest efficiently after treatment with microtubule poisons, their loss may create lesions that are not monitored correctly by the checkpoint. In *Xenopus* extracts, BubR1 forms a complex with the adenomatous polyposis coli-EB1 dimer that is potentially enhanced by microtubule attachment (104). This mechanism may be conserved in mammals, as adenomatous polyposis coli can be phosphorylated by human BubR1 *in vitro* (36). Importantly, adenomatous polyposis coli also interacts with and is a substrate for Bub1 (36), and careful dissection of the individual kinase contribution to adenomatous polyposis coli phosphorylation and function in mitosis will be necessary to resolve this issue.

Interestingly, instability in microtubule attachments caused by BubR1 depletion can be suppressed upon Aurora B inhibition, suggesting that BubR1 and Aurora B activities may counteract each other (48). A significant body of work has demonstrated that Aurora B activity is required for destabilizing erroneous attachments, such as merotelic configurations, through phosphorylation of microtubule binding factors such as MCAK and Hec1 (15, 16, 38, 47). In yeast, the Aurora B orthologue Ipl1 also phosphorylates Mad3 to regulate the checkpoint response to lack of tension at kinetochores (39). However, the Ipl1 phosphorylation sites on Mad3 are not conserved in higher eukaryotes, and the interplay between Aurora B and BubR1 remains to be fully explored. Nevertheless, as Aurora B and BubR1 kinase activities have opposing functions with respect to attachment stability, it will be interesting to test whether key Aurora B substrates such as MCAK and Hec1 are shared with BubR1.

CONCLUDING REMARKS

The progress achieved in our understanding of Bub1 and Mad3/BubR1 in recent years has revealed their remarkably pleiotropic behavior and has begun to unravel their role in APC/C^{Cdc20} inhibition at the molecular level (see Fig. 2 for a summary). Numerous questions nonetheless remain unanswered. In particular, the significance of kinase activity to the checkpoint is unclear. Broadly speaking, the checkpoint is functional without either Bub1 or BubR1 kinase activity, but target phosphorylation may play a role in ensuring accurate chromosome attachment. Catalytic activity appears to be more important in certain cell lines and developmental contexts for prolonging the checkpoint response. While incomplete protein inactivation in complementation assays can explain these observations, we postulate that variations arise as a reflection of the inherent differences in the efficiency of the microtubule capture machinery in the various cell lines. This in turn is a function of the number of chromosomes that must be captured relative to cell volume and microtubule density in a particular cell or organism. In the future, the development of small-molecule inhibitors will be critical for advancing our understanding of Bub1 and BubR1, as it would enable unprecedented spatial and temporal control of kinase activity. In light of their dual role in both congression and the checkpoint, these compounds would also facilitate evaluation of Bub1 and BubR1 inhibition as a means to curb cancer cell proliferation (34). Ultimately, identification of biologically relevant substrates will be necessary to answer some of the lingering questions.

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