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Review

# Mps1 kinase functions in mitotic spindle assembly and error correction

Victor M. Bolanos-Garcia [1,*]

**The protein kinase Mps1 (also known as TTK) is a central component of the mitotic spindle assembly checkpoint (SAC), an essential self-monitoring system of the eukaryotic cell cycle that ensures accurate chromosome segregation by delaying the onset of anaphase until all chromosomes are properly bioriented on the mitotic spindle. Mps1 kinase is an important upstream regulator of the SAC and its recruitment to kinetochores critical for initiating SAC signaling. This review discusses the current understanding of Mps1 essential functions in the SAC, the emerging details of Mps1 role in error correction to safeguard genome stability, and the therapeutic potential of Mps1 inhibition for the treatment of cancer types associated with aberrant SAC signaling and chromosome segregation defects.**

## Highlights

Mps1 promotes mitotic checkpoint complex assembly and the establishment of bioriented chromosome attachment to spindle microtubules.

Mps1 is a multidomain protein kinase that harbors protein motifs mediating its function in the SAC. These motifs are highly conserved during evolution, but with some important deviations that are species specific.

The molecular description of Mps1 roles in SAC signaling and error correction has enhanced our understanding of the mechanisms that govern cell division. Defects in cell division result in gross chromosome segregation errors and genome instability.

Inhibition of Mps1 catalytic activity to interfere with mitosis progression in tumor cells is a promising therapeutic window for the treatment of aggressive types of cancer.

Mps1 may also be a suitable target for the treatment of infections caused by diverse pathogens, such as fungi.

## Mps1, an evolutionarily conserved protein kinase

During mitosis, **kinetochores** (see Glossary) on each pair of replicated sister chromatids must establish bioriented attachments to microtubules emanating from opposite spindle poles [1]. The multidomain protein kinase monopolar spindle 1 (Mps1), also known as TTK, is the most upstream regulator of the **spindle assembly checkpoint (SAC)**, the signaling system responsible for monitoring errors in chromosome attachment to spindle microtubules, thus delaying cell cycle progression until these errors are corrected [2,3]. This protein kinase was originally identified in budding yeast as being required for the proper duplication of the **spindle pole body**, hence the acronym 'Mps1' [4]. Despite some species-specific details, Mps1 function in the SAC is evolutionarily conserved in different eukaryotic organisms, ranging from fungi to vertebrates to green plants [5–10].

This review discusses what is known of the function, structure, and regulation of the central mitotic checkpoint protein kinase Mps1/TTK. Particular attention is given to the establishment of higher-order complexes mediated by Mps1 and how such interactions contribute to kinetochore recruitment and error correction to ensure proper **chromosome segregation** and genome stability. Finally, there is a discussion of the true potential of Mps1 as a bona fide molecular target for cancer therapies.

## Roles of Mps1 in mitosis

In most eukaryotes, Mps1 kinase activity is required for the recruitment of Bub1, BubR1, Mad1, Mad2, and CENP-E to unattached kinetochores and for SAC signaling [11,12]. Moreover, Mps1 kinetochore localization drives phosphorylation of multiple Met-Glu-Leu-Thr motifs in the kinetochore protein **Knl1** [kinetochore-null 1; also known as blinkin (Bub-linking) in humans, Spc105 in budding yeast and frogs, and Spc7 in fission yeast] to recruit the SAC protein components Bub1 and Bub3 to unattached kinetochores [13–16] (Figure 1A).The subsequent interaction of kinetochore-bound Bub1 with BubR1 recruits BubR1/Bub3 to the kinetochore [17,18]. Following Bub1-Bub3 and BubR1-Bub3 kinetochore recruitment, Mps1 promotes the interaction between Bub1 and the Mad1:C-Mad2 complex, which is essential for checkpoint signaling, by phosphorylating Bub1. Mps1 also contributes to the regulation of the SAC via a conformational switch in Mad2 to enhance MAD2 open-close (O–C) conversion, promoting the formation of the complex between

[1]Faculty of Health and Life Sciences, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford OX3 0BP, UK

*Correspondence:
vbolanos-garcia@brookes.ac.uk
(V.M. Bolanos-Garcia).

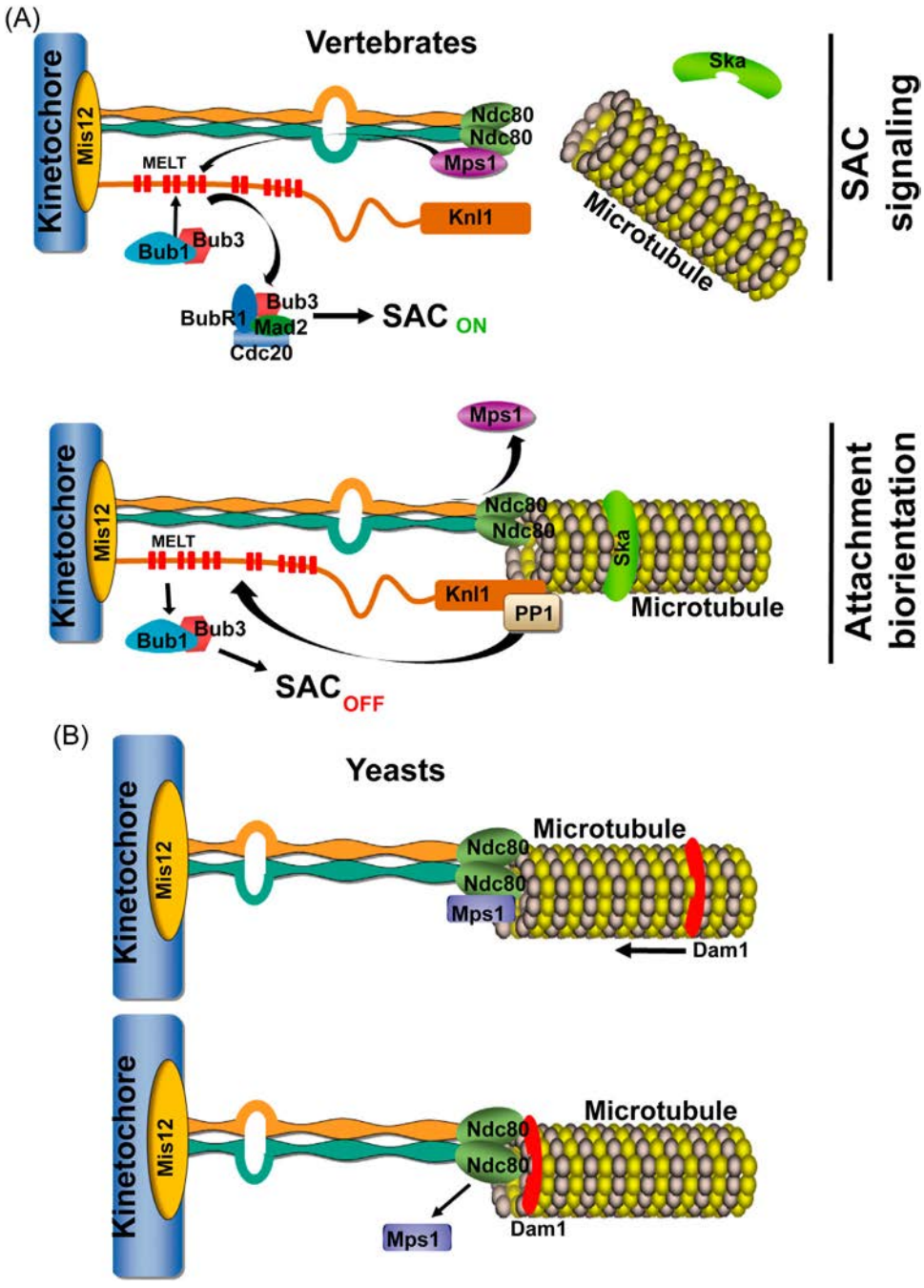



Figure 1. Schematic model of monopolar spindle 1 (Mps1) functions in the spindle assembly checkpoint (SAC). Mps1 is recruited to the kinetochore to allow mitotic checkpoint signaling, with the Ndc80 complex being its main kinetochore receptor in both yeast and vertebrates. Although the function of the SAC is evolutionarily conserved, there are important species-specific singularities. (A) In human cells, in concert with the Ndc80 network, the Ska complex contributes to establishing stable kinetochore–microtubule interactions. Once the SAC is satisfied (i.e., all chromosomes undergo bipolar attachment and are aligned at the center of the cell), inhibition of the anaphase-promoting complex or cyclosome (APC/C) is released. In this scenario, kinetochore-associated protein phosphatase 1 (PP1) inactivates Mps1 to ensure efficient SAC silencing. (B) In yeast cells, upon formation of bioriented kinetochore microtubule attachments (upper

*(Figure legend continued at the bottom of the next page.)*

## Glossary

**Allosteric inhibitor:** a class of enzyme inhibitor that binds to a specific site on an enzyme other than the active site, known as the allosteric site.

**Amphitelic attachment:** a condition also known as 'biorientation' where sister kinetochores on chromosomes are attach to microtubules emanating from opposite poles of the mitotic spindle.

**Anaphase-promoting complex or cyclosome (APC/C):** a multisubunit E3 ubiquitin ligase protein assembly that targets various cell-cycle proteins for their timely degradation by the proteasome. An interplay of allosteric activation by Cdc20 and inhibition by the MCC directly controls APC/C ubiquitin ligase activity in mitosis.

**Aneuploidy:** a condition in which premature separation of sister chromatids results in the loss or gain of chromosomes in daughter cells. Aneuploidy constitutes a prevalent form of genetic instability observed in many types of human cancer.

**Biorientation:** the process by which sister chromatids are attached to both poles of the mitotic spindle. This is achieved through microtubules emanating from different microtubule organizing centers attached to kinetochores of sister chromatids.

**Centromere:** a specialized region of chromosomes where sister chromatids remain joined.

**Chromosome segregation:** the process by which sister chromatids are distributed to two daughter cells.

**Kinetochore:** a large, protein-rich complex that assembles onto the centromeric region of chromosomes only during mitosis and serves as a signaling platform as well as the site of interaction of spindle microtubules.

**KNL1:** a human protein that acts as a central component of the KNL1-Mis12-Ndc80 (KMN) network. In humans, it is also referred to as Blinkin, CASC5, and AF15Q14; it is also known as KNL-1 in worms, Spc105 in yeast, and Spc105R in flies.

**Nonsense mutation:** a change in DNA that causes the termination of translation earlier than expected resulting in a truncated protein. This is a common type of mutation in humans and in other animals that results in the expression of a partially functional or nonfunctional protein.

Cdc20 and Mad2 in a closed conformation (C-Mad2) [19–21] (Figure 1A). In humans, mutation of the phosphorylation sites of Mad1 by Mps1, including residue T716, compromises the formation of the Mad1-Mad2 complex and the maintenance of a viable checkpoint *in vivo* [20–22]. Definition of the specific function of the other Mad1 residues phosphorylated by Mps1 remains obscure. Moreover, Mad1 T716 phosphorylation by Mps1 also contributes to Mad1 binding to Cdc20's N-terminal region, and this is a critical step for *in vivo* catalysis of the **mitotic checkpoint complex (MCC)** [23], the multiprotein assembly that acts as an **allosteric** regulator of the **anaphase-promoting complex or cyclosome** (**APC/C**) E3 ubiquitin ligase activity toward cyclin B1 and securin that controls mitotic exit [24–27]. Although Bub1 is recruited after Mps1-mediated phosphorylation of Knl1 to the kinetochore, Bub1 is also a protein substrate of Mps1. In budding yeast, phosphorylation of the Bub1 residue T455 by Mps1 appeared to be sufficient to promote Bub1-Mad1 complex formation [16,21]. Ultimately, the phosphorylation of these central protein components of the SAC by Mps1 and the subsequent concerted association of Mad1-Cdc20, Mad2-Cdc20, and Bub1-Mad1 enable the formation of the MCC and the regulation of SAC silencing [12,20,23,27–30].

Mps1 importance in SAC signaling is further evidenced by the number and diversity of phosphorylation substrates, which include yeast Ndc80 (residues S4, T38, T248, and T252), yeast Dam1 (residue S221), and yeast Spc29 (residues T18, T159, S187, and T240). A comparative analysis of these Mps1 phosphorylation substrates and other protein substrates of Mps1 not involved in SAC signaling, such as human borealin (residue T94), yeast Cdc31 (residue T110), human Cep215 (residue S613), human MCAK (residue T87), and human Nup98 (residue S591), reveals that they share a consensus motif: E/D/N/Q at –2 position [31].

## Structure of Mps1

Considerable advances have been made in the definition of the structure and function of individual domains of Mps1. Across multiple species, Mps1 is organized in two main domains: an N-terminal α-helical region encompassing the **tetratricopeptide repeat (TPR) motif** and a C-terminal region harboring a catalytically active kinase domain (Figure 2A). The TPR motif is flanked by an N-terminal end (NTE) region and a C-terminal extension (CTE) region present in human Mps1 and conserved in other vertebrates (Figure S1 in the supplemental information online). Secondary structure analysis of the NTE region predicted the presence of a long α-helix (amino acids 9–25) and a second short α-helix (amino acids 50–58) in this region [32]. The crystal structure of human Mps1 N-terminal region (residues 55–210) [33] has been shown to harbor a triple tandem arrangement of the TPR motif. Interestingly, similar to Mps1, the SAC proteins Bub1 and BubR1 (it should be noted that at least in the human, the latter protein acts as a pseudokinase) also contain a conserved N-terminal triple tandem TPR motif that shares high structural similarity [33–35] (Figure 2B). However, TPR Mps1 does not define a shallow groove that in TPR1 Bub1 and TPR BubR1 is essential for binding to Knl1 (Figure 2C). The Mps1 kinase domain presents canonical features, namely an N-terminal lobe consisting of a series of antiparallel β-sheets and a conserved α-helix and a C-terminal lobe that is predominantly helical. Multiple phosphorylation sites span the Mps1 polypeptide chain (Figure 2D).

## Recruitment of Mps1 to the kinetochore

The recruitment of Mps1 to the kinetochore is an essential requirement for Mps1 roles in the SAC and accurate spindle microtubule attachment (Figure 1) [36,37]. At least in budding yeast, the

**P+1 loop:** a small motif residing immediately downstream of the activation loop of a kinase domain. The P+1 loop received its named for its role in contacting the residue immediately C-terminal to the phosphorylated tyrosine (the P+1 position of the residue) in the substrate. The P+1 loop is implicated in recognizing the residues next to tyrosine to be phosphorylated in the substrate.

**Spindle assembly checkpoint (SAC):** the evolutionarily conserved surveillance mechanism of eukaryotic organisms that ensures high-fidelity chromosome segregation during cell division. In mitosis and meiosis, the SAC prevents the onset of anaphase until all chromosomes are properly attached to spindle microtubules.

**Spindle pole body:** a large, multilayered, plaque-like structure present in fungi that is the functional equivalent of the centrosome in higher eukaryotes. The spindle pole body acts as a microtubule organizing center where microtubules are assembled.

**Syntely:** an attachment defect in which both sister kinetochores are attached to the same pole.

**Tetratricopeptide repeat (TPR) motif:** a protein motif defined by a helix-loop-helix, in which the consensus 34–amino acid sequence contains small hydrophobic residues at positions 8, 20, and 27; large hydrophobic residues at positions 4, 7, 11, and 24; and one α-helix-breaking residue, typically a proline, at position 32.

panel), the Dam1 complex acts as a crucial factor for kinetochore end-on attachments. Dam1 displaces Mps1 from its binding site on the Ndc80 complex (lower panel) and thereby promotes anaphase onset, in which sister chromatids separate and are pulled toward opposite poles of the cell. In this cartoon, the yeast Knl1 orthologue Spc105 is not shown for simplicity. Abbreviation: MELT, Met-Glu-Leu-Thr.

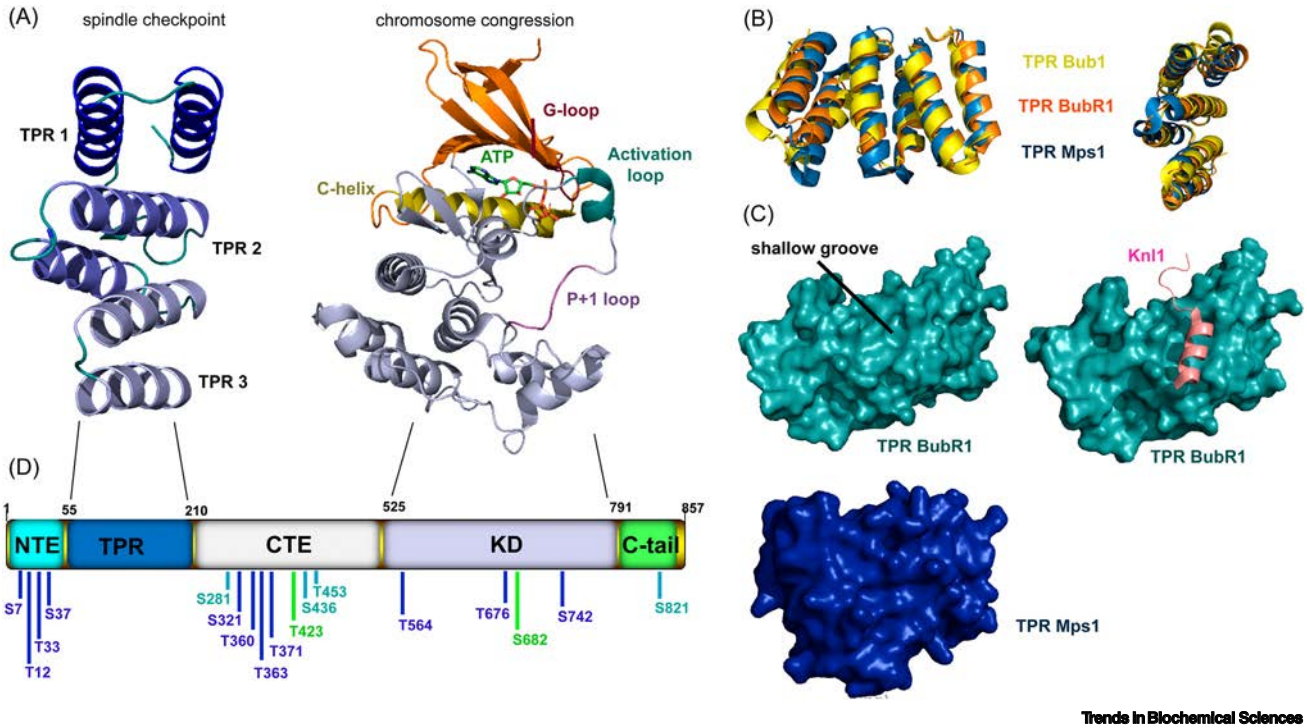



Figure 2. Overall monopolar spindle 1 (Mps1)/TTK protein structure. (A) Mps1 domain organization. The TPR-containing domain consists of a triple tandem of the TPR motif (TPR1, TPR2, and TPR3). The TPR Mps1 3D structure corresponds to the PDB ID 4H7Y. Key functional domains of Mps1 kinase domain such as the G-loop, the activation loop, and the **P+1 loop** are mapped onto the 3D structure (PDB ID 6GVJ). (B) Structure superposition of human TPR Mps1 (PDB ID 4H7Y), human TPR-Bub1 (PDB ID 2LAH), and human TPR-BubR1 (PDB ID 2WVI) reveals an overall high 3D structural similarity. (C) TPR Mps1 lacks a shallow groove present in Bub1 and BubR1 and involved in Knl1 binding. (D) Mapping of phosphorylation sites in human Mps1. Phosphorylation sites conforming to a putative Plk1, Cdk1/MAPK, and Aurora B consensus motif are shown in purple, cyan, and green, respectively. Residue T676 corresponds to an autophosphorylation site that is mapped onto the activation loop of the kinase domain. Abbreviations: CTE, C-terminal extension; C-tail, C-terminal end region; KD, kinase domain; NTE, N-terminal end; TPR, tetratricopeptide repeat.

latter process implicates Ndc80 phosphorylation [38–40] by Mps1 via multisite interactions with the Ndc80 complex [34,40–42] (Figure 1). Key yeast Mps1 autophosphorylation sites are located in a region mediating Mps1 binding to the Ndc80 complex [43]. Two different crystal structures show that the Ndc80 complex binds Mps1 and Ipl1 at an overlapping site (Figure 3). The two short Mps1 and Ipl1 regions implicated in Ndc80 binding are predicted to be of low structural complexity on their own. However, the crystal structures of the Ndc80-Mps1 and Ndc80-Ipl1 complexes show that the Mps1 and Ipl1 fragments adopt an α-helical structure upon Ndc80 binding, most likely involving a disorder to order conformational transition, a feature previously observed in the Knl1–TPR Bub1 and Knl1–TPR BubR1 interactions [44,45]. Moreover, one 3D structure model based on *de novo* structural predictions using the neural network–based model AlphaFold [46,47] suggests that Dam1 binds to the same Mps1 and Ipl1 binding site, a notion that is supported by pulldown experiments and peptide binding measurements [48]. Importantly, site-specific mutants mapped onto this Ndc80 protein binding region exhibit severe chromosome segregation errors and viability defects, confirming the biological significance of the interaction and the functional importance of the aforementioned binding interface. Whether other regions of the Ndc80 complex or components of the Knl1-Mis12-Ndc80 signaling axis contribute to Mps1 binding remains to be established. There is evidence that Aurora B phosphorylation toward the N-terminal region of the Ndc80 protein subunit significantly enhances Mps1-Ndc80 complex formation [49,50]. Hence, in addition to Mps1 regulation through phosphorylation by Cdk1 and self-phosphorylation, an interplay of Mps1 and Aurora B kinase activities are

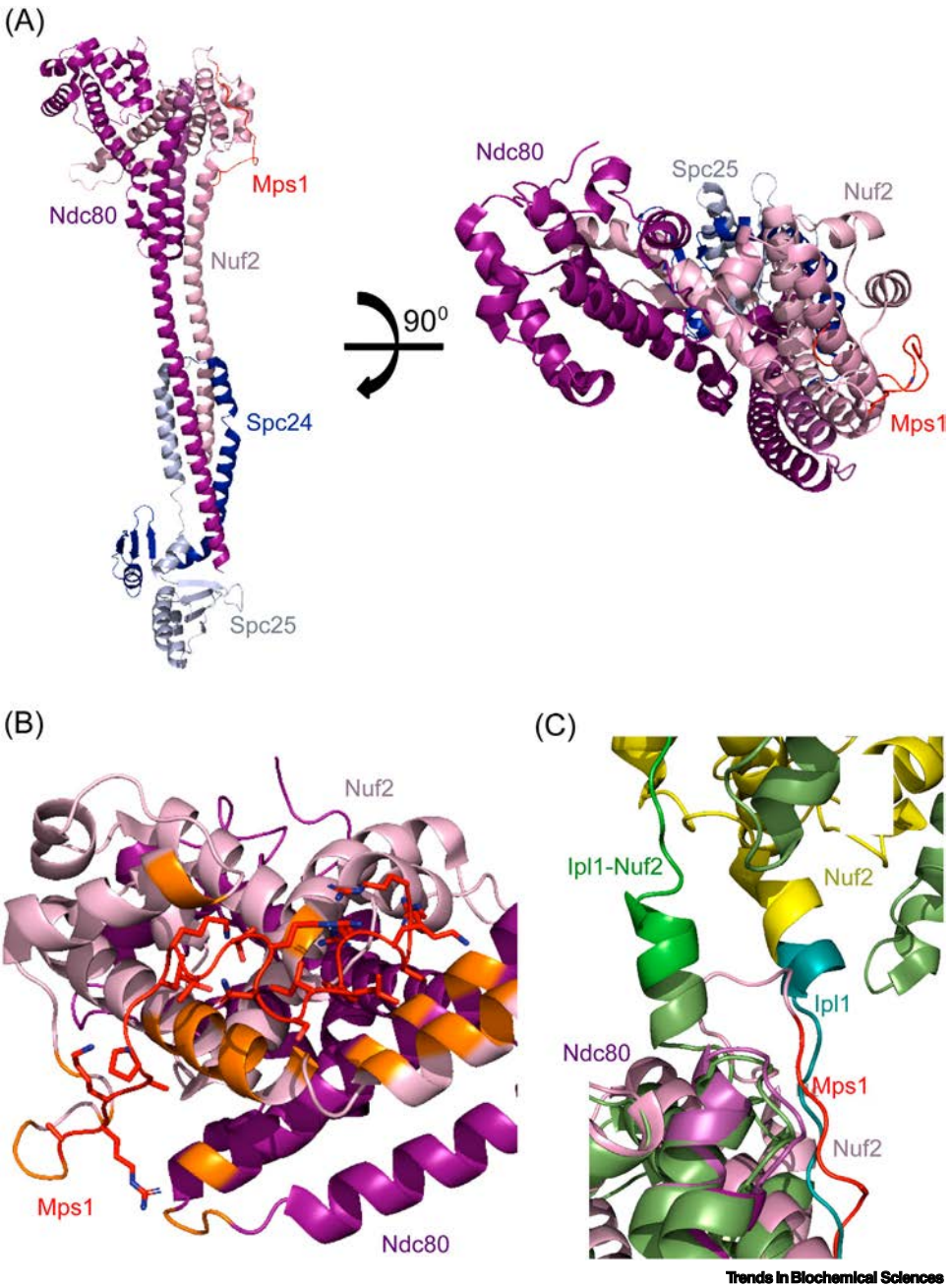



Figure 3. 3D structure of monopolar spindle 1 (Mps1)-Ndc80 complex. (A) Two orthogonal views of an Ndc80 short protein construct (e.g., Ndc80 dwarf) in complex with a short fragment of yeast Mps1 (residues 137–171) (PDB ID 8V10). The figure shows the overall topology of the complex and the association of Mps1 with the globular head of the Nuf2 subunit of the Ndc80 complex. (B) Close-up of the mode of binding of the Mps1 fragment (shown in red) to the Ndc80 complex. The Ndc80 subunit of the Ndc80 dwarf complex is shown in magenta and the Nuf2 subunit fused to Mps1 in light pink. The Nuf2 residues implicated in Mps1 binding are highlighted in orange. (C) Structural superposition showing that an overlapping Ndc80 site binds Mps1 137–171 (PDB ID 8V10) and N-terminal Ipl1 (yeast Aurora B), residues 26–39 and 45–59 (PDB ID 8V11) [48]. The structures provide insights into a communication hub that ensures uniform bipolar attachment and anaphase onset.

coordinated in time and space to monitor kinetochore attachment to spindle microtubules during cell division.

Deletion of the gene region that encodes the Mps1 N-terminal region leads to chromosome segregation errors, increased chromosome instability, and an attenuated SAC response, confirming the importance of this region for Mps1 recruitment to the kinetochore [34,51]. The NTE/CTE and TPR have been shown to cooperate to enhance the binding affinity of Mps1 for the Ndc80 complex, a central component of the outer-layer kinetochore composed of the Spc24, Spc25, Ndc80, and Nuf2 proteins [34,37,52]. Interestingly, the NTE and TPR regions are absent in yeast, but the CTE of both yeast and human Mps1 can bind to the corresponding Ndc80 complex despite the low sequence similarity of the binding motifs [49,53]. These features reflect important species-specific variations of an evolutionarily conserved checkpoint mechanism of eukaryotic organisms [53,54]. In addition to this notion, independent kinetochore localization studies in cells expressing human Mps1 fragments fused to both N- and C-terminal YFP together with bimolecular fluorescence complementation assays showed that different Mps1 fragments varying in length bind to the Ndc80 complex with different binding affinities. N-terminal Mps1 constructs comprising the NTE region (amino acids 1–61), the TPR-containing region (amino acids 62–192), and a C-terminal fragment (amino acids 193–300) all showed significantly weaker kinetochore localization signal intensities than a fragment encompassing the N-terminal Mps1 residues 1–300 [32]. Other studies showed that the N-terminal kinetochore-binding domain contributes to regulation of Mps1 levels at unattached kinetochores [33,55] and that the interaction between the Mps1 NTE and TPR motif promotes Mps1 release from the kinetochore [34,56]. Taken together, these investigations further confirm the requirement of the Mps1 N-terminal region for the functional integrity of the SAC and faithful mitotic progression.

Different molecular mechanisms have been proposed to describe the recruitment of Mps1 to the kinetochore. One model postulates direct competition of Mps1 with microtubules, which would prevent Mps1 binding to kinetochores already attached to microtubules [49,52]. An alternative model proposes that an interplay of kinase and phosphatase activities (Aurora B and PP1 and PP2A-B56, respectively) modulates Mps1 recruitment and release at unattached kinetochores [57,58]. HeLa cells expressing GFP-Mps1 fusions and treated with calyculin A, a potent inhibitor of the protein phosphatases PP2A and PP1, resulted in a significantly higher amount of Mps1 in bioriented end-on attached kinetochores compared with untreated cells, adding support to the notion that inhibition of these phosphatases stabilizes Mps1 localization to attached kinetochores [57].

### Post-translational modifications and protein interactions impact Mps1 recruitment

In human Mps1, the residues S281 and S821 have been identified as Cdk1 substrate sites [31]. These amino acid residues are mapped onto the CTE region and the C-terminal tail, respectively. However, whether Mps1 S281 phosphorylation by Cdk1 is critical for Mps1 kinetochore localization remains controversial [59,60]. The use of a Mps1 kinase-dead mutant showed a significantly stronger kinetochore signal than that of native Mps1, suggesting that the kinase activity of Mps1 plays a role in the regulation of its localization to the kinetochore [32,61]. Furthermore, an Mps1 fragment encompassing the kinase domain and C-terminal tail region that is highly conserved and predicted to harbor two α-helices involved in Mps1 dimerization enhanced Mps1 kinetochore localization, indicating an important role of the C-terminal region in Mps1 kinetochore recruitment [32].

A quantitative mass spectrometry study of HeLa cells treated with the Mps1 kinase inhibitor SP600125 identified 12 Mps1 autophosphorylation sites *in vivo*: S7, T12, T33, S37, S80, S321, S363, T371, S382, T676, T686, and S837 [31]. Mps1 autophosphorylation of residue

T676, which is mapped onto the kinase activation loop (Figure 2D), is required for a robust SAC response *in vivo* [61,62]. Similar to the case of human Mps1, *in vitro* experiments with isolated yeast kinetochore particles showed that yeast Mps1 autophosphorylation induced its release from the kinetochore [63]. Despite these advances, the functional relevance of some phosphorylation residues is controversial. For instance, it has been argued that phosphorylation of human Mps1 residues T12 and S15 is required for Mps1 kinetochore recruitment [64], whereas others have reported that phosphorylation of such residues is dispensable for this process [65]. Elucidation of the contribution of autophosphorylation sites in Mps1 kinetochore recruitment in different species remains to be fully established. Additional post-translational modifications of Mps1 such as SUMOylation may contribute to the regulation of Mps1 function in the SAC [66]. However, clarification of important details of the role of SUMOylation and other post-translational modifications on Mps1 functions during cell division requires more investigation.

Several pieces of evidence support Mps1 functions as a homodimer *in vivo* [51,67]. A Mps1 fragment encompassing only the N-terminal region showed a weaker dimerization activity than the full-length protein, evidencing that the Mps1 C-terminal region can greatly enhance Mps1 dimerization [32]. Moreover, the use of an Mps1 deletion mutant encompassing the C-terminal residues (516–857) showed that Mps1 kinase activity negatively regulated Mps1 dimerization [32]. Whether this is due to the adoption of a conformation that relieves Mps1 dimerization or the prevention of Mps1 dimer formation following autophosphorylation demands additional studies.

## Role in error correction

In eukaryotic organisms ranging from yeast to humans, during normal cell division, sister kinetochores on chromosomes attach to microtubules from opposite spindle poles, leading to **biorientation** (a condition also known as '**amphitelic attachment**') [68]. Different types of kinetochore–microtubule attachment errors can occur in this process, resulting in aberrant chromosome segregation. These include single unattached kinetochores (monotely), single kinetochores with microtubule attachments to both spindle poles (merotely), and sister kinetochore attachment to the same spindle pole (**syntely**) [68]. The correction of these attachment defects is crucial for accurate chromosome segregation and genome stability [69].

Several reports have addressed the error correction function of Mps1 separately from its roles in the SAC. Although it is technically difficult to untangle this Mps1 function from other error correction pathways due to the intrinsic redundancy of error correction mechanisms, unequivocal insights into the role of Mps1 in the correction of kinetochore–microtubule attachment errors arise from recent biochemical, biophysical, and structural biology studies of yeast kinetochores. For instance, the components of the spindle microtubule attachment system DASH/Dam1 and Ndc80 complexes were noted to physically interact with Mps1 and Ipl1 (the yeast orthologue of Aurora kinase B) to integrate SAC signals at the kinetochore [43,48,70,71]. The DASH/Dam1 complex is an essential component of the yeast kinetochore that assembles into a microtubule-encircling ring [71,72]. Phosphorylation of Dam1 by Mps1 is required for efficient association of the yeast kinetochore with the plus end of a microtubule [73]. The Ndc80 complex functions as an organizing platform that recruits Mps1, Dam1, and Ipl1 in an asynchronous fashion [43,48,70]. When kinetochores are not under tension, Mps1 binding to the Ndc80 complex activates the SAC and phosphorylates protein substrates of the outer kinetochore to promote the release of improper attachments. Once stable kinetochore end-on attachments are formed, Dam1 displaces Mps1 from the Ndc80 complex, which silences the SAC (Figure 1B).

During error correction, Mps1 kinetochore localization via the Ndc80 complex avoids direct competition between Mps1 and Dam1. In this way, the generation of bipolar end-on attachments can

be coupled to the tension-sensitive error correction machinery [43,48,70]. The completion of error correction involves dephosphorylation of Dam1 by PP1 and Mps1 proteolytic degradation through APC/C polyubiquitination in anaphase [74]. Similar to the function of the Dam1 complex in yeast cells, the metazoan-specific Ska complex ensures that kinetochores remain attached to microtubule ends during depolymerization, a process that steers mitotic chromosome movement (Figure 1A). Phosphorylation of the Ska complex contributes to the destabilization of improper attachments that is required for accurate chromosome segregation. Mps1 plays a role in the regulation of the Ska complex through phosphorylation of residue S34 of the Ska3 subunit (also called C13orf3 or RAMA1) [54]. Such an Mps1-regulated site in Ska3 is mapped onto the hinge region of the Ska complex, which adopts a W-shape (Figure S2 in the supplemental information online).

It should also be noted that, at least in budding yeasts, Mps1 play a key role in shugoshin recruitment, a protein involved in the regulation of biorientation. Mps1 phosphorylates a serine-rich motif of Sgo1, which is essential for shugoshin-condensin interaction at the **centromere** and the establishment of biorientation [75–77]. This role of Mps1 further complicates the recognition of direct error correction targets of Mps1 from indirect ones. So, the question is how exactly do concerted, specific protein–protein interactions contribute to Mps1 roles in error correction? This question remains to be clarified. Moreover, it will be important to establish how exactly Mps1 contributes to error correction in other species. The recent identification of mutants of Mps1 that are fully proficient in bipolar spindle assembly and able to separate Mps1 function in error correction and the SAC appears promising to clarify these details [43].

Despite the gaps in knowledge and the species-specific features outlined above, the picture emerging is that Mps1 autophosphorylation, together with an intricate network of intra- and intermolecular protein–protein interactions, control Mps1 kinetochore localization and contribute to error correction to ensure the high fidelity of chromosome segregation during cell division.

## Mps1 in disease

Cancer has become the second leading cause of death in the human population, contributing to one in six deaths worldwide. According to the World Health Organization, the most common cancers are breast, lung, prostate, stomach, and colorectal [78]. Many human tumors are shown to make too much Mps1, which contributes to uncontrolled cancer cell growth and the accumulation of additional mutations in cancer cells [79]. An early study showed that Mps1 mRNA was found to be overproduced in 38% of all breast tumors and in 85% of triple-negative tumors [80]. Mps1 amplification not only occurs in hormone-responsive and non–hormone-responsive breast cancer subtypes [81] but also facilitates an **aneuploidy**-tolerant state in breast cancer cells [82]. Moreover, Mps1 overproduction correlates with worse prognosis and poor survival in tumors of different tissue origin, including breast, bladder, thyroid, pancreas, and brain [82–86]. Therefore, blocking Mps1 from working can lead to cancer cell death and to halting tumor growth. The observed promotion of cell death in neuroblastoma [87] and the potent activation of STING and STAT1 pathways in KRAS and STK11 comutated (KL) non–small cell lung cancer to restore KL tumor cells' immunogenicity following Mps1 inhibition [88] support this view.

The impact of Mps1 inhibition is complex, affecting the formation of Cdc20 complexes, the induction of cell cycle arrest in $G_1$ phase, and multinucleation [89,90].

The functional screening of diverse Mps1 inhibitors that belong to different chemical classes against a panel of 66 genetically characterized cell lines derived from different tumors revealed that cell lines harboring activating mutations in the CTNNB1 gene, which encodes β-catenin, a key signaling regulator of the Wnt pathway, were up to five times more sensitive to the inhibitors

than CTNNB1 wild-type cell lines. Because mutations in the CTNNB1 gene are comparatively frequent in liver and endometrial cancer, two types of tumor that produce high amounts of Mps1, it is possible that mutant CTNNB1 can be used as a drug response biomarker, helping to stratify patient groups that are most likely to respond to Mps1 inhibition therapy [91].

The online database Catalogue of Somatic Mutations in Cancer (COSMIC) [92] lists 613 individual entries for Mps1, and, in all the cases, Mps1 was reported to be overexpressed. COSMIC also lists a number of *Mps1* gene deletions, insertions, and point and silent mutations that are associated with aneuploidy, chromosome instability, and cancer. The distribution of such *Mps1* gene mutation types is summarized in Figure 4A. About 43% of missense and 3% of nonsense

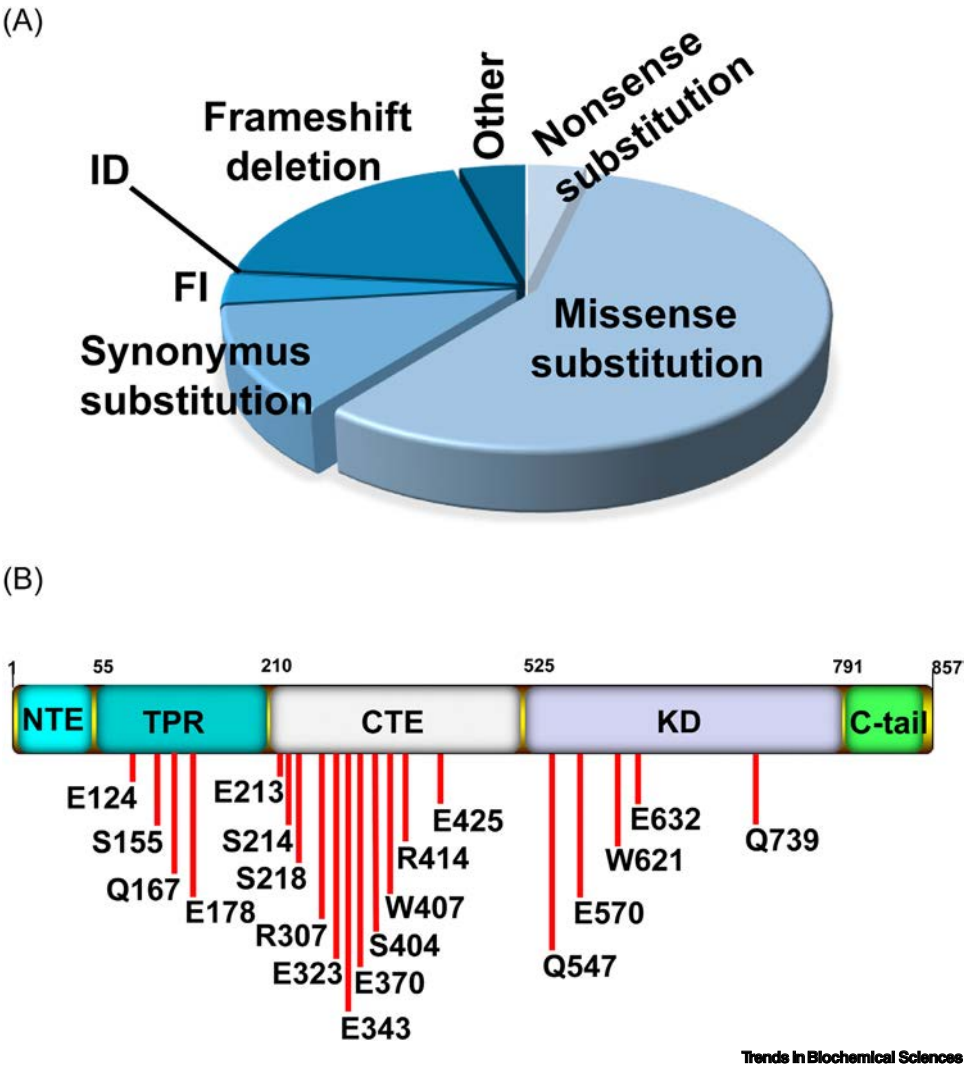
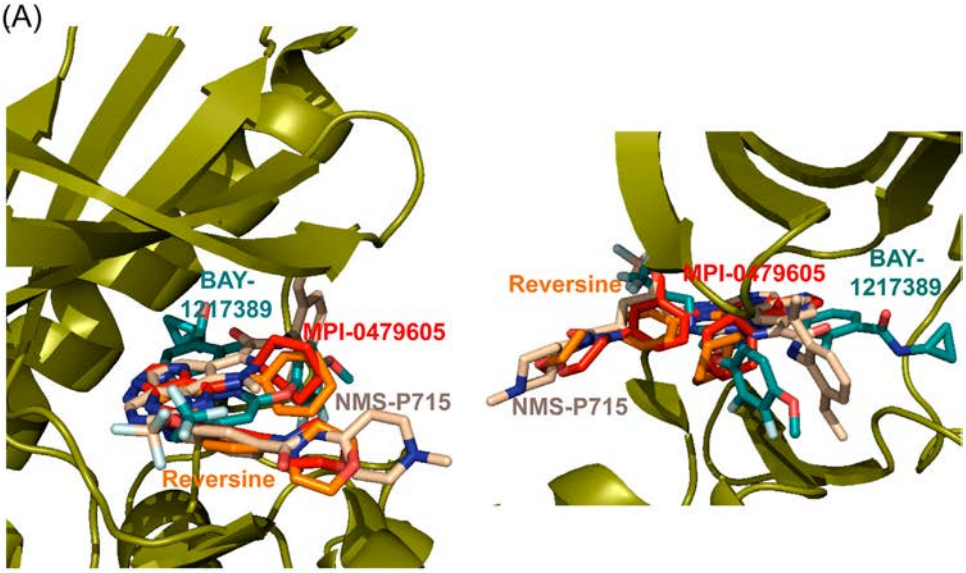
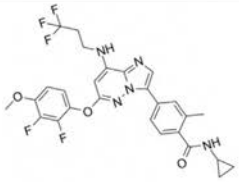
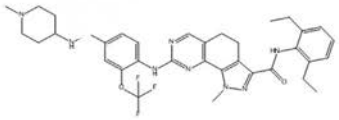
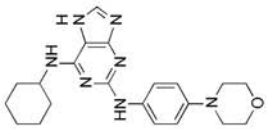
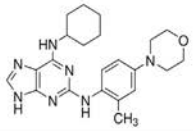



Figure 4. Human monopolar spindle 1 (Mps1) mutations associated with cancer. (A) Pie chart showing the distribution of the various types of Mps1 mutations. Figure adapted from the Catalogue of Somatic Mutations in Cancer (COSMIC) database [92]. (B) Mapping of nonsense mutations in full-length Mps1 reported in COSMIC [92]. It is worth noting that the majority of the nonsense mutations are located in the intermediate region, which is predominantly disordered. The majority, if not all, of the nonsense mutations are expected to result in the production of proteins that are misfolded and/or have a low stability. Abbreviations: FI, frameshift insertion; ID, inframe deletion.

(A)




(B)

| PDB ID | Inhibitor name | Chemical structure | IC50 (nM) |
|---|---|---|---|
| 5NAD | BAY-1217389 |  | 110 |
| 2X9E | NMS-P715 |  | 1800 |
| 5LJJ | Reversine |  | 240 |
| 5N7V | MPI-0479605 |  | 440 |

Trends in Biochemical Sciences

Figure 5. Monopolar spindle 1 (Mps1) kinase is a bone fide drug target. Because the blockade of mitosis progression is an effective strategy to induce mitotic catastrophe, ultimately resulting in cell death, for nearly two decades, inhibition of the catalytic domain of Mps1 with small-size compounds has been explored for cancer therapy. As a result of these efforts, a variety of ATP analogues that belong to different chemical classes, including methylbenzamide, purine, pyrimidine, and quinazoline, have been developed for the treatment of cancer, with some compounds reaching clinical trials in humans. Although to date no Mps1 kinase inhibitor has been approved for use in the clinic, this spindle assembly checkpoint regulator remains a pivotal target for drug development. Moreover, inhibition of Mps1 kinase activity has been

*(Figure legend continued at the bottom of the next page.)*

substitutions were identified on COSMIC from a pool of 792 unique tissue samples. It can be anticipated that some missense mutations will alter the function of the protein. Interestingly, although gene fusions are a cause of cancer that occurs in rare and common cancer types, to date, no gene fusions involving Mps1 have been reported in COSMIC. Structural information allows the mapping onto the protein surface of some of the *Mps1* mutations that have been identified and associated with chromosome instability and cancer progression. About 55% of the *Mps1* **nonsense mutations**, which are expected to result in protein misfolding and a low stability, can be mapped onto the CTE region of Mps1 (Figure 4B).

### Targeting Mps1 with small molecules

ATP binding competitors that belong to different chemical classes and exhibit different potency have been developed to interfere with Mps1 activity in cancer cells (Figure 5 and Table 1). Because the catalytic domain of protein kinases is structurally conserved, the selective binding to a specific protein kinase target but not the more than 500 other kinases that define the human kinome may appear very challenging. However, a diversity of conformational dynamics changes has been observed among human kinases [93], a feature that may be exploited for the design of specific kinase inhibitors. Indeed, the vast majority of small protein kinase inhibitors approved by the FDA correspond to ATP-binding competitors [94]. Although currently no drugs to treat cancer that target Mps1 catalytic activity have been approved for use in the clinic, some Mps1 kinase inhibitors have been advanced to clinical trials: CFI-402257, BAY-1161909, BOS-172722, BAY-1217389, and S-81694 [95–97]. All these inhibitors have been tested for triple-negative breast cancer. Of the five compounds, the first is still ongoing clinical trials, the second has been terminated, and the other three have been completed [96]. Phase I studies of orally administered BAY-1217389 and BAY-1161909 in combination with intravenous paclitaxel were encouraging, with both compounds showing good efficacy and moderate adverse effects, such as anemia and fatigue. However, due to a strategic decision, only the latter compound was recommended to be advanced to phase II studies. In addition to triple-negative breast cancer, the inhibitor CFI-402257 has been tested for the treatment of hepatocellular carcinoma and advanced solid tumors (ClinicalTrials.gov identifier NCT02792465) [98].

However, the fact that the Mps1 inhibitor BAY-1217389 showed a synergistic effect in combination with either low doses of taxanes or a CENP-E inhibitor to promote tumor cell death via the enhancement of cell division errors [99,100] and that the combinatorial use of chitosan nanoparticles, reversine (a small-molecule inhibitor of Mps1 and Aurora B that induces premature exit from mitosis, aneuploidy, and cell death) [101], and X-ray irradiation (2 Gy) can sensitize breast cancer cells [102] suggest that new therapeutic opportunities involving Mps1 targeting remain to be explored. These include the use of innovative Mps1 inhibitors alone or in combination with drugs currently in use in the clinic, such as taxanes and mitotic blockers [99,100], the concomitant use of innovative Mps1 small-size inhibitors with X-ray irradiation and/or with new nanomaterials for the sensitization of cancer cells and/or as drug carriers [102], as well as emerging therapeutic approaches involving small-size compounds of diverse chemical classes, covalent inhibitors, and targeted proteolysis chimeras [103–107]. These are bifunctional molecules that recruit the ubiquitin-proteasome system for the selective proteolytic degradation of a protein of interest. Indeed, a recent report described the targeted degradation of Mps1 in human colorectal cancer cells and a xenograft mouse model of colorectal cancer [108].

proposed to develop innovative drugs to treat fungal infections in animals, including humans, and plants of high social and commercial value, such as rice. This is important because current drugs used to treat pathogen fungal infections exhibit undesired side effects in the host.

Table 1. 3D structures of Mps1 in complex with small-sized inhibitors reported in the Protein Data Bank (PDB)

| PDB ID | Encompassing residues | Resolution (Å) | Inhibitor name |
|---|---|---|---|
| 2X9E | 514–828 | 3.10 | NMS-P715 |
| 2ZMD | 510–857 | 2.88 | SP600125 |
| 3GFW | 519–808 | 2.74 | Pyrrolopyridine |
| 3H9F | 519–808 | 2.60 | Pyrimido-diazepine |
| 3HMO | 510–809 | 2.40 | Staurosporine |
| 3HMP | 510–809 | 2.30 | Quinazoline |
| 3VQU | 516–820 | 2.40 | Aminobenzamide |
| 3W1F | 516–820 | 2.70 | Benzenesulfonamide |
| 3WYX | 516–820 | 2.90 | Nicotinonitrile |
| 3WYY | 516–820 | 3.05 | Phenyl-acrylamide |
| 3WZJ | 516–820 | 2.75 | N-cyclopropyl-benzamide |
| 3WZK | 516–820 | 2.30 | Pyrazine-benzamide |
| 4BHZ | 519–808 | 2.85 | Pyrrolopyridine |
| 4BI0 | 519–808 | 2.84 | Pyrrolopyridine |
| 4BI1 | 519–808 | 2.70 | Pyrrolopyridine |
| 4BI2 | 519–808 | 3.11 | Pyrrolopyridine |
| 4C4E | 519–808 | 2.60 | Pyrrolopyridine |
| 4C4F | 519–808 | 2.36 | Pyrrolopyridine |
| 4C4G | 519–808 | 2.65 | Pyrrolopyridine |
| 4C4H | 519–808 | 2.80 | Pyrrolopyridine |
| 4C4I | 519–808 | 2.65 | Pyrrolopyridine |
| 4C4J | 519–808 | 2.50 | Pyrrolopyridine |
| 4CV8 | 519–808 | 3.00 | Aminopyridine |
| 4CV9 | 519–808 | 2.50 | Aminopyridine |
| 4CVA | 519–808 | 2.50 | Aminopyridine |
| 4D2S | 514–795 | 2.50 | Compound 23 |
| 4JS8 | 515–795 | 1.94 | 401348 |
| 4JT3 | 515–795 | 2.20 | 400740 |
| 4O6L | 515–795 | 2.38 | Indazole-5-carboxamide |
| 4ZEG | 515–795 | 2.33 | Pyrazole pyrimidine |
| 5EH0 | 519–794 | 2.18 | Pyrimidine |
| 5EHL | 519–808 | 2.66 | Pyrimidine |
| 5EHO | 519–808 | 2.18 | Pyrimidine |
| 5EHY | 519–808 | 2.26 | Pyrimidine |
| 5EI2 | 519–808 | 2.67 | Pyrimidine |
| 5EI6 | 519–808 | 2.01 | Pyrimidine |
| 5EI8 | 519–753 | 2.17 | Pyrimidine |
| 5LJJ | 519–808 | 3.00 | Reversine |
| 5MRB | 519–808 | 3.00 | Cpd-5 |
| 5N7V | 519–808 | 2.52 | MPI-0479605 |
| 5N84 | 519–808 | 2.30 | Mps-BAY2b |
| 5N87 | 519–808 | 2.29 | NTRC 0066-0 |

*(continued on next page)*

Table 1. (continued)

| PDB ID | Encompassing residues | Resolution (Å) | Inhibitor name |
|---|---|---|---|
| 5N93 | 519–808 | 2.10 | TC-Mps1-12 |
| 5N9S | 519–808 | 2.30 | BAY 1161909 |
| 5NA0 | 519–808 | 2.90 | Pyrimido-indolizine |
| 5NAD | 519–808 | 2.80 | BAY 1217389 |
| 5NTT | 519–797 | 2.75 | NMS-P715 |
| 6B4W | 515–795 | 2.90 | Compound 23 |
| 6H3K | 519–794 | 2.48 | BOS172722 |
| 6N6O | 515–795 | 2.60 | Pyrrolopyrimidine benzamide |
| 6TN9 | 515–806 | 2.60 | Compound 16 |
| 6TNB | 515–806 | 2.65 | BAY 116909 |
| 6TNC | 515–806 | 2.30 | Compound 46 |
| 6TND | 515–806 | 2.58 | BAY 1217389 |
| 7CHM | 515–795 | 2.65 | Compound 8 |
| 7CHN | 515–795 | 2.40 | Compound 9 |
| 7CHT | 515–795 | 2.40 | Compound 30 |
| 7CIL | 515–795 | 2.30 | Compound 7 |
| 7CJA | 515–795 | 2.49 | Compound 28 |
| 7CLH | 515–795 | 2.90 | Compound 19 |
| 7LQD | 519–808 | 1.95 | RMS-07 |

## Outstanding questions

How exactly do Aurora B and Mps1 activities coordinate the kinetochore-microtubule state?

How do Mps1 roles contribute to tension sensitivity?

How does Mps1's activity at kinetochores contribute to biorientation?

How exactly do autophosphorylation and other post-translational modifications such as SUMOylation regulate Mps1 functions in the SAC?

Are there other amino acid residues of the Ndc80 and/or DASH/Dam1 complex binding sites for Mps1? If yes, how do such interactions affect the dynamics of the complex?

How exactly do phosphorylation/dephosphorylation reactions control Mps1 displacement from the kinetochore?

Are there other phosphorylation targets of Mps1 when bound to the kinetochore?

## Concluding remarks

The engagement of Mps1 in multiple protein–protein interactions through various motifs located across the polypeptide chain highlights its remarkable plasticity of functions. This knowledge provides molecular insight into the recognition mechanism that mediates Mps1 localization to the kinetochore. The emerging structural details of Mps1 alone and in complex with other proteins and the generation of separation-of-function mutants provide a foundation for defining Mps1 roles in the SAC, chromosome biorientation, and error correction. Furthermore, given the potential benefit of targeting the SAC in anticancer therapy, the Mps1 kinase domain has been used extensively in structure-guided drug design. Future work could aim to gain a deeper understanding of how Mps1 contributes to translating the imbalance of force at kinetochore microtubules into an effective SAC response and to advance into the clinic new therapeutics targeting Mps1 for cancer treatment and possibly a diversity of infections caused by pathogenic organisms (see Outstanding questions). In summary, the transient localization of Mps1 to end-on attached kinetochores importantly contributes to microtubule release, SAC activation, and error correction.

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### Declaration of interests

No interests are declared.

### Supplemental information

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