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The MPS1 Family of Protein Kinases

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Abstract

MPS1 protein kinases are found widely, but not ubiquitously, in eukaryotes. This family of potentially dual-specific protein kinases is among several that regulate a number of steps of mitosis. The most widely conserved MPS1 kinase functions involve activities at the kinetochore in both the chromosome attachment and the spindle checkpoint. MPS1 kinases also function at centrosomes. Beyond mitosis, MPS1 kinases have been implicated in development, cytokinesis, and several different signaling pathways. Family members are identified by virtue of a conserved C-terminal kinase domain, though the N-terminal domain is quite divergent. The kinase domain of the human enzyme has been crystallized, revealing an unusual ATP-binding pocket. The activity, level, and subcellular localization of Mps1 family members are tightly regulated during cell-cycle progression. The mitotic functions of Mps1 kinases and their overexpression in some tumors have prompted the identification of Mps1 inhibitors and their active development as anticancer drugs.

Keywords

TTK; spindle checkpoint; mitosis; kinetochore; cell cycle

1. INTRODUCTION

Protein kinases are critical regulators of cell division. Apart from the cyclin-dependent kinases (CDKs), which are considered the master regulators, a suite of additional conserved kinases control progression through mitosis, including Polo, Aurora, Bub, NEK/NimA, and MPS1 kinases. Collectively, these have been called the mitotic kinases because of the widely conserved nature of their functions in mitosis. Here, we review the MPS1 family of protein kinases, which are still being discovered, dissected, and potentially exploited for therapeutics owing to their critical functions in the control of mitosis.

DISCLOSURE STATEMENT

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1.1. Discovery and Initial Characterization

The MPS1 gene (monopolar spindle) was first identified in the budding yeast, *Saccharomyces cerevisiae*. The original mutant allele, *mps1-1*, causes yeast cells to fail at a restrictive temperature in spindle pole body (SPB) assembly (the yeast centrosome) (1), a critical cell-cycle event that is necessary to form a bipolar spindle. The *MPS1* gene was first cloned as an essential gene encoding an apparent protein kinase by Poch et al. (2). It was named *RPK1*, but *MPS1* is used because it was the first published name. Lauze et al. (3) demonstrated that glutathione *S*-transferase (GST)-tagged Mps1 was indeed a protein kinase. GST-Mps1 exhibited robust autophosphorylation, as well as substrate phosphorylation of several common in vitro kinase substrates. Mps1 was able to phosphorylate serines/threonines and tyrosines, suggesting that it is a dual-specificity protein kinase, but thus far, no biologically relevant substrate is known to be phosphorylated on tyrosine by Mps1.

Phenotypic analysis of the original yeast *MPS1* mutants identified key functions of the kinase. As noted above, *mps1-1* was first discovered because of a defect in yeast SPB duplication, leading to an aberrant monopolar spindle. It was also observed that *mps1-1* mutant cells failed to arrest in mitosis with the monopolar spindle defect unlike other mutants defective in SPB duplication [*kar1* (4), *cdc31* and *mps2* (1)]. A subsequent study demonstrated that this phenotype was because of the role of Mps1 in the spindle checkpoint (5). In addition, Hardwick et al. (6) showed that Mps1 overexpression caused mitotic arrest by triggering the spindle checkpoint and identified the checkpoint protein, Mad1, as the first Mps1 substrate. All of the original *MPS1* alleles caused defects in both SPB duplication and in the spindle assembly checkpoint at a restrictive temperature (7). Interestingly, electron microscopic examination of the SPBs in these various strains revealed that Mps1 is required for multiple steps of SPB assembly.

Prior to cloning of the yeast *MPS1* gene, Mps1 orthologs from humans [phosphotyrosine-picked threonine kinase/threonine and tyrosine kinase (PYT/TTK) aka hMPS1] (8, 9) and mice [esk (EC STY kinase aka MmMps1)] (10) had been discovered. Intriguingly, these kinase genes were identified in screens using antiphosphotyrosine antibodies that tested expression libraries for protein kinases that autophosphorylate on tyrosine residues. Indeed, these kinases phosphorylate serine, threonine, and tyrosine residues in vitro, offering the initial evidence that this is a family of dual-specificity protein kinases. Also, it was observed that both mRNA and protein levels of Mps1/TTK are readily detectable in all proliferating human cells and tissues but are markedly reduced or absent in resting cells and in tissues with a low proliferative index (11).

1.2. Mps1 Kinase Features

MPS1 kinase family members are ~85–95 kDa and have conserved C-terminal kinase domains. The N-terminal domains of the family members appear unrelated, and they lack any unifying motif [such as the Polo box observed in the Polo family of kinases (12)]. Nonetheless, the kinase domain is distinctive enough to identify family members (<http://www.signaling-gateway.org/molecule/query?afcsid=A000882&type=blast&adv=latest>). The crystal structure of the kinase domain reveals interesting features that are discussed below.

1.3. MPS1 Distribution and Diversity

MPS1 kinase genes are found in most eukaryotes. Interestingly, there is no well-documented case of a genome containing paralogs of an *MPS1* gene. However, *MPS1* isoforms generated by alternative splicing have been predicted in humans and observed in mice (10). Orthologs are easily identified in fungi, vertebrates, and invertebrates, like *Drosophila*, as well as in plants, including the ancient plant lineage of lycophytes (*Selaginella moellendorffii*), diatoms (*Phaeodactylum tricornutum*), and alga (*Chlamydomonas*) (13). Although no validated *MPS1* has been identified in the nematode *Caenorhabditis elegans*, there are orthologs in the pathogenic nematode (*Globodera*), as well in flat and round worms.

2. MPS1 FUNCTIONS

MPS1 kinases have multiple roles in mitosis that we briefly survey here. The most widely conserved and prominent function of these kinases is to ensure proper biorientation of sister chromatids on the mitotic spindle at kinetochores, and this function involves the spindle checkpoint. Along with being implicated in other cellular processes, MPS1 kinases also function from the earliest steps of mitosis, including spindle pole duplication, to the latest steps of mitotic exit and cytokinesis.

2.1. Spindle Pole Assembly

As described above, the budding yeast *MPS1* gene was identified by a mutation that is defective in SPB (centrosome) duplication. A collection of *MPS1* alleles revealed that the kinase acts in multiple steps of the duplication pathway (7), and the kinase has been shown to phosphorylate numerous SPB components. These include Spc29 and Spc42, which are fungus specific, whose assembly and stability are controlled by Mps1 phosphorylation (14–16). The more widely conserved SPB components that are Mps1 substrates include centrin (Cdc31) (14), the γ -tubulin complex component Spc98 (17), and the Spc110 tether that holds the γ -tubulin complex (Tub4, Spc98, Spc97) to the SPB (18). Centrin (Cdc31) is a small, EF-hand calcium-binding protein (19), and its phosphorylation by Mps1 influences its interaction with a binding partner (14). Phosphorylation of Spc98 is only found on the nuclear pool of the γ -tubulin complex, possibly influencing its interaction with Spc110 (17). Likewise, Mps1 phosphorylation of Spc110 (in conjunction with phosphorylation by Cdc28; the yeast CDK) is required for interaction with Spc97 (18). Interestingly, both Mps1 and Cdk phosphorylate most of these substrates such that combinatorial control appears to be the rule (20, 21).

Mps1 is localized to SPBs in yeast (15), and the mammalian enzymes are localized to centrosomes (Figure 1). Overexpression of mammalian Mps1 leads to overduplication of the centrosomes, and overexpression of a kinase-inactive allele blocks centrosome duplication (reviewed in Reference 22). Despite these results, RNAi experiments have produced contradictory results concerning a requirement for Mps1 in centrosome duplication (22). Recently, Mps1 was deleted from human cell lines using cre-lox, and these cells were capable of centrosome duplication (23). Similarly, the fission yeast ortholog, Mph1 (the *Schizosaccharomyces pombe* Mps1 homolog), is not required for SPB duplication (24), nor is the *Drosophila* ortholog, Ald, required for centrosome duplication (25). Finally, C.

elegans appears to lack an MPS1 ortholog and can execute centriole and centrosome duplication.

Nonetheless, Mps1 influences centrosome duplication in human cells (reviewed by Reference 22). Furthermore, the centrosomal levels of hMps1 are exquisitely controlled, separately from other pools of the kinase (discussed below). Additionally, hMps1 centrosomal substrates, such as mortalin (26) and centrin 2 (27), have been identified. The phosphorylation of centrin 2 is required for its ability to stimulate centriole (the microtubule-based structural core of the centrosome) assembly (27). Remarkably, the major site of centrin 2 phosphorylation by hMps1 is T118 (27), which is the site analogous to the yMps1-phosphorylated T110 on the yeast centrin Cdc31 (14). These results suggest a deeply conserved regulatory event.

2.2. Kinetochores and the Spindle Assembly Checkpoint

MPS1 kinases have universally conserved functions at kinetochores. In yeast, Mps1 was implicated in the spindle checkpoint (5, 6) and was later shown to be localized to kinetochores (15). The spindle checkpoint monitors the correct bipolar attachment and tension of all chromosomes to the mitotic spindle. The cells are held at metaphase until every chromosome is properly attached; then the cells can proceed into anaphase. The molecular target of the checkpoint is the anaphase-promoting complex (APC), a ubiquitin ligase that targets mitotic cyclins and other proteins for destruction, allowing the cells to segregate their chromosomes. The APC is controlled by the Cdc20 activator, which in turn can be inactivated by the checkpoint protein Mad2. Mad2 is activated in the course of cycling on and off unattached kinetochores. A variety of other checkpoint proteins act with Mad2, both on and off of the kinetochore, to inhibit Cdc20 activity and therefore inhibit APC.

Xenopus Mps1, XIMps1, was the first vertebrate ortholog implicated in the spindle checkpoint and localized to kinetochores (28). *Xenopus* oocyte extracts require XIMps1 for mitotic arrest and for the recruitment of Mad2 and other checkpoint proteins to the kinetochore. Human Mps1 is also found at kinetochores and is required for the activation and maintenance of the spindle checkpoint (29, 30). These results have been repeated in several recent studies using a variety of tools, including depletion of hMps1, conditional deletion of the hMps1 gene, and small-molecule inhibition of the native or engineered forms of the kinase (reviewed in Reference 31). In most systems, kinetochore localization of Mad2 also requires active hMps1, although Mad2 appears not to be a hMps1 substrate.

Kinetochore localization of checkpoint proteins is important for their function. In *Xenopus*, Cenp-E localization to kinetochores requires XIMps1 (28), and CENP-E has been identified as an Mps1 substrate in vitro (32). However, this result, and similar dependencies on Mps1 for kinetochore localization of various checkpoint proteins, has not been universally observed. Lan & Cleveland (31) carefully document the various discrepancies and propose that they arise from the use of various Mps1-inactivating methods and different cell types. Some of these studies, but not all, show the loss of several checkpoint proteins from the kinetochore when Mps1 is inactivated, consistent with work in *Xenopus*. Similarly, the Mps1 overexpression-induced arrest in yeast is dependent on the function of each of the

checkpoint proteins. Collectively, these results suggest Mps1 is near the top of the checkpoint-signaling pathway. However, the complexity of the data indicates that a linear pathway may be too simple a model for the checkpoint. An alternative view is that hMps1 is a linchpin in a checkpoint network such that the absence of Mps1 activity disrupts several checkpoint activities, leading to catastrophic failure of the network and other spindle-related functions (31). These predictions are complicated by the fact that Mps1 likely has several substrates in this pathway or network. Already known Mps1 checkpoint substrates are Mad1 (in yeast), Cenp-E, and Mps1 itself (6, 32, 33).

Interestingly, Mps1 can act in the checkpoint without being present at the kinetochore. The overexpression of yMps1 is capable of imposing a mitotic arrest in *ndc10-1* strains (34), which normally do not arrest because the mutation destroys the kinetochore and obviates its ability to act in checkpoint signaling. A similar phenomenon has been observed in human cells using a truncated allele of hMps1 that does not localize to kinetochores but retains the catalytic domain (23). This allele can still activate the mitotic checkpoint complex (35), which inhibits Cdc20 during mitosis. Mps1 also contributes to the formation of an interphase APC inhibitor that shares components with the mitotic checkpoint complex, such as Mad2 and BubR1 (23). Although these proteins can inhibit Cdc20 in vitro without Mps1 (36), their association in vivo is dependent on Mps1 activity (23). Indeed, Mps1 is so critical to controlling normal mitotic progression that cells lacking Mps1 activity transit mitosis faster than cells with the activity (reviewed in Reference 31).

Prior to checkpoint signaling, both the Aurora B and Mps1 kinases are required for the proper bipolar spindle attachment of chromosomes. These kinases are involved in resolving syntelic attachments in which both kinetochores of replicated sister chromatids are attached to the same pole instead of their correct bipolar attachment (23, 37–41). The dependency relationship between Aurora B and Mps1 in vertebrates is a point of contention. In some studies, Aurora B activity is reduced upon reduction of Mps1 activity (42, 43), placing Aurora B downstream of Mps1. One reported mechanism for this dependency is via phosphorylation of the chromosomal passenger protein Borealin, which influences Aurora B activity (42, 44). However, other studies, using various hMps1 inhibitors or inhibitable hMps1 alleles, found that Aurora B activity was unchanged by reducing Mps1 activity (23, 39, 40). Indeed, two of these studies (39, 40) have shown instead that reducing Aurora B activity results in reduced hMps1 at kinetochores, similar to findings in *Xenopus* extracts (45). Saurin et al. (46) report that Aurora B and the kinetochore component Hec1/hNdc80 are both required for Mps1 recruitment to the kinetochore, a precondition that can be circumvented by tethering Mps1 to the kinetochore. Furthermore, the kinetochore recruitment of Mps1 is necessary for timely activation of Mps1 in mitosis. These results place Aurora B upstream of hMps1 in the spindle checkpoint pathway, as seen in yeast (37). However, the yeast kinetochore protein Dam1 is a substrate of both Ipl1 [the yeast Aurora B kinase (47)] and yMps1 (48), suggesting that the kinases collaborate in controlling kinetochore attachment. Similarly, in reviewing recent findings, Lan & Cleveland (31) also suggest that shared substrates may explain some of the complexity in Aurora B and Mps1 interactions. Their candidate substrate for analysis is CENP-E, which is phosphorylated by both Aurora B (49) and Mps1 (32). Ndc80/Hec1 should be considered as well, as the yeast

Ndc80 protein is an Mps1 substrate (50), and Hec1/hNdc80 is a substrate of Aurora B (51). Finally, Ipl1 and the chromosomal passenger complex in yeast can act in a pathway distinct from γ Mps1, Sgo1 (shugoshin), and Bub1 in processing syntelic attachments (52).

The collective Mps1 functions in chromosome segregation are sufficient to make the enzyme essential in most organisms [though *S. pombe* Mps1 (Mph1) is not essential (24)]. The use of inhibitable alleles in budding yeast (53) and in human cells (reviewed in Reference 31) reveals that cells die without Mps1 function, likely because of severe aneuploidy. In zebrafish, Mps1 (called nightcap) has also been found to be especially crucial for the very rapid and extensive cell proliferation during tissue regeneration, presumably because it prevents excess aneuploidy (54–56).

2.3. Other Signaling Pathways

Several lines of evidence implicate Mps1 in the genotoxic stress response. Genotoxic stress, such as DNA damage, causes tumor cells to arrest at G2/M or G1, or to commit apoptosis depending on the status of p53. Mps1 influences these responses through multiple mechanisms. Upon exposure to X-ray or UV irradiation, robust G2/M arrest of HeLa cells requires the activity of Mps1, which has been attributed to direct interaction between Mps1 and CHK2/Rad53/Cds1. Mps1 has been shown to phosphorylate CHK2 at Thr68 (57). CHK2 reciprocates Mps1's action by phosphorylating Mps1 on Thr288 and increasing its stability, thereby creating a positive feedback loop for CHK2 Thr68 phosphorylation (58). Disruption of this positive feedback attenuates the DNA damage checkpoint at G2/M arrest (57). The Bloom syndrome protein (BLM) is another Mps1 target in the DNA damage pathway (59). BLM phosphorylation at Ser144 by Mps1 promotes its association with and phosphorylation by Polo-like kinase. Ser144 phosphorylation is important for sustained mitotic arrest in response to microtubule poisons and for accurate chromosome segregation (59).

Mps1 may also be involved in another facet of genotoxic stress response by regulating phosphorylation and subcellular localization of c-Abl. c-Abl is phosphorylated at Thr735, and pThr735-c-Abl normally localizes in cytosol, but it enters the nucleus upon exposure to oxidative stress (60). Mps1 has been identified as the Thr735 kinase, and phosphorylation of c-Abl at Thr735 enhances its association with 14-3-3 protein and cytoplasmic sequestration (60). It has been proposed that Mps1 phosphorylates c-Abl under normal and oxidative stress conditions.

The function of Mps1 in genotoxic response depends on the status of p53. Mps1 is required for the apoptotic response in p53 null cells exposed to the topoisomerase I inhibitor (CPT-11) (61), and Mps1 suppression partially overrides CPT-11-induced cell death. In the presence of functional p53, however, CPT-11 treatment leads to growth arrest without mitotic entry (61). This result suggests that cancer cells with high levels of Mps1 but defective p53 checkpoint pathways are susceptible to DNA damage-induced cell death. There is also a direct link between Mps1 and p53. In response to microtubule poisons, p53 is stabilized and phosphorylated at Thr18, which has been attributed to Mps1 (62). Phosphorylation of p53 by Mps1 may contribute to the postmitotic checkpoint, which arrests

cells in G1, thereby preventing further increase in DNA content and genome polyploidization (62).

Mps1 has also been implicated in modulating other cellular signaling responses. Depolymerization of microtubules by nocodazole results in phosphorylation of Smad2 and Smad3 proteins at the C-terminal SSXS motif, a site normally targeted by TGF- β type I receptor (63, 64). Interestingly, this event requires Mps1 and is independent of TGF- β type I receptor (64). Mps1 interacts with Smad4 and phosphorylates Smad2/3 in vitro. Phosphorylation of Smad2 at the C-terminal SSXS motif and at the linker region increases significantly during mitosis. However, the significance of Smad2 mitotic phosphorylation remains unclear. Also, Mps1 has been shown to be a negative regulator of the MAP kinase (MAPK) pathway in yeast (65), although the specific Mps1 target(s) have not been identified.

2.4. Cytokinesis

Mps1 RNAi in human cells led to the appearance of multinucleated cells and to the proposal that Mps1 is involved in the exit from mitosis and/or cytokinesis (66). Hints of this from budding yeast include localization of Mps1 to the bud neck (67) and interaction with a Mob1 (Mps1 one binder) protein (68). Mob1 binds and activates the Dbf2 protein kinase, and the complex acts in the mitotic exit network (69). Although the interaction of yMps1 and Mob1 is not understood, Mps1 is inactivated as cells exit mitosis (70). A more direct link between Mps1 and cytokinesis comes from the discovery of an hMps1-binding partner and substrate, MIP1 (Mps1 interacting protein 1), which is a component of the actin cytoskeleton (71). MIP1 RNAi led to the accumulation of multinucleate cells and disorganization of the actin cytoskeleton. Live-cell recordings revealed a spindle rocking phenotype indicative of difficulties in organizing the cytokinetic furrow. It is not known how MIP1's interaction with, or phosphorylation by, hMPS1 affects its function.

2.5. Meiosis

Disruption of MPS1 function in meiotic yeast cells (72), during meiosis I in mouse oocytes (73), female meiosis in *Drosophila melanogaster* (74, 75), and germ cell production in zebrafish (76), leads to severe chromosome missegregation and aneuploidy. These defects may all arise from the Mps1 mutants' failing to maintain the spindle checkpoint and/or to properly attach chromosomes on the meiotic spindle, suggesting similar Mps1 functions in meiosis as detailed above in mitosis. In fact, Straight et al. (72) were able to demonstrate defects in both meiosis I and meiosis II segregation. Much of this work was done with hypomorphic alleles (25, 72, 76), suggesting that meiotic chromosome segregation is particularly sensitive to disruption in Mps1 activity. For instance, during meiosis I, hypomorphic alleles of the *Drosophila* Mps1 gene, *Ald*, destroy the metaphase pause, which normally leaves the spindles ample time to segregate the nonexchange chromosomes, leading to their loss in *ald* mutant flies (25).

Separate from spindle-related functions, Mps1 has been implicated in other meiotic and germ cell formation functions. Yeast Mps1 is required for both rounds of meiotic SPB duplication (72). Also in yeast, Mps1 is required for the postmeiotic event of spore

formation. This developmental pathway depends on a transcriptional regulatory network and the function of a MAPK cascade. Mutant strains in *MPS1* retain the normal function of the transcription regulatory network, but the *mps1⁻* phenotypes resemble defects in the Ste20-family kinase Sps1, such that Mps1 may function with this kinase to control specific aspects of the spore formation program (72).

XlMps1 has been implicated in CSF (cytostatic factor) function, which causes meiosis II metaphase arrest in eggs by inhibiting APC. Two distinct pathways, Mos dependent and CDK2/cyclin E dependent, contribute to the CSF arrest. XlMps1 is required for the CDK2/cyclin E-mediated arrest of cycling extracts (77). Paradoxically, XlMps1 activity is reduced at CSF arrest and must be restrained for extracts to exit CSF arrest. This regulation, as well as Mps1 synthesis, is dependent on CDK2/cyclin E and is associated with different electrophoretic versions of XlMps1, suggesting control by phosphorylation (77). Finally, *Drosophila Ald* has been implicated in hypoxia-induced arrest and the arrests of polar bodies, both of which may reflect a checkpoint function (74). Interestingly, *Drosophila Ald*, along with Polo kinase, is found in novel filamentous structures in oocytes that appear at the end of prophase and are maintained until egg activation (25, 78).

3. MPS1 STRUCTURE, ENZYMOLOGY AND INHIBITORS

3.1. Structure of the Mps1 Catalytic Domain

Several groups have solved the structure of the hMps1 kinase domain with very good agreement (79–82). The Mps1 kinase domain adopts the typical protein kinase bilobe architecture. The N-terminal small lobe consists of a standard five-stranded β -sheet and an α C helix, a canonical feature seen in many protein kinases (Figures 2a and 3). In addition, Mps1 contains an extra β -strand (β 0) at the N terminus of the small lobe, which, together with part of β 1, covers the twisted β -sheet (Figure 2a). The two lobes are joined by a hinge loop (Glu603-Gly605) at the back of the active-site cleft. The C-terminal large lobe shows a standard kinase structure, composed of a two-stranded β -sheet (β 6 and β 7) adjacent to the small lobe, seven α -helices, the catalytic loop, and the activation loop. The loop between helices α EF and α F (700–708) and the C-terminal tail are disordered (795–857). All of the reported Mps1 catalytic domain structures adopt an inactive conformation, as indicated by incorrect positioning of the α C helix, which prevents ion pairing between the conserved α C glutamate (Glu571) and the active-site lysine (Lys553), the unstructured activation loop, and the inactive conformation of the P+1 loop (684–688). In two structures, a polyethylene glycol molecule, which is a widely used precipitant in protein crystallization, is present as a ring surrounding the catalytic lysine (Lys553). Even though polyethylene glycol is artificially introduced into Mps1 by the crystallization conditions, its presence created a secondary pocket unseen in other kinases, a feature that could be exploited for inhibitor design. The Mps1 kinase domain has been cocrystallized with ATP (83). However, the ATP did not significantly alter the kinase domain conformation in that the Mps1-ATP structure is indistinguishable from the apo or inhibitor-bound conformations. This result suggests that ATP binding is insufficient for switching the kinase to an active conformation, raising tantalizing questions about the active kinase conformation (83).

3.2. Regulation of Mps1 Activity by Phosphorylation

The Mps1 C-terminal catalytic domain undergoes autophosphorylation and is active toward exogenous substrates (33, 80, 81, 84, 85). Initial structure characterization efforts focused on either dephosphorylated, kinase-defective mutants or the normal kinase complexed with small-molecule inhibitors (79, 80). These structures reveal the expected inactive kinase domain conformation, indicated by an unstructured activation, a P+1 loop, and an incorrect α C helix position (Figures 2*a* and 3) (79, 80). Surprisingly, structures of the wild-type kinase reveal that the catalytic domain still adopts an inactivate conformation, despite extensive autophosphorylation at nine different sites (81). The active catalytic domain may be quite heterogeneous owing to extensive posttranslational modifications; this makes it challenging to obtain crystals of the highly active enzyme. Although dephosphorylated enzymes can be prepared, the enzyme reactivates when ATP is introduced (33, 79). The puzzle remains as to why the active catalytic domain alone or in complex with ATP does not lead to an active enzyme conformation.

Mps1 undergoes extensive autophosphorylation *in vitro*. Mps1 isolated from mitotic HeLa cells or insect cells were also phosphorylated at numerous sites (84–88). Phosphorylation occurs predominantly at Ser/Thr sites, although Tyr phosphorylation is also observed *in vitro* (33, 79). Among a myriad of phosphorylation sites, Thr676 and Thr686 are observed to have significant effects on kinase activity (33, 79, 80, 84, 85, 89). Thr676 lies in the activation loop, whereas Thr686 is on the P+1 loop. Mutation of the Thr676 residue to Ala reduces Mps1 transphosphorylation kinase activity by sevenfold. Interestingly, this mutation causes only a 1.4-fold reduction in autophosphorylation (33). Nevertheless, Thr676 phosphorylation is required for Mps1 to function optimally in yeast and in human cells (33, 84, 85). Supporting the theory that autophosphorylation increases kinase activity, kinetic analysis of Mps1 phosphorylation revealed a lag phase in product formation that is eliminated by preincubation with cold ATP (80, 89). Therefore, Thr676 phosphorylation is likely a priming event for kinase activation. Without an active Mps1 structure, we can only speculate about how phosphorylation stabilizes the activation loop. Mps1 lacks the basic RD pocket, which is referred to as a catalytic loop between the β 6 and β 7 strands featuring highly conserved Arg (R) and Asp (D) residues. The basic RD pocket, present in many protein kinases regulated through activation loop phosphorylation, directly interacts with the phospho residue in the activation loop, causing a switch to an active conformation. In the place of the RD pocket, it has been proposed that the Mps1 pThr676 phospho group may interact with one of the three lysine residues in the disordered loop between α EF and α F (700–708) (84). Confirmation or repudiation of this hypothesis awaits the availability of an active Mps1 catalytic domain structure.

The Thr residue at the beginning of the P+1 loop is invariant in numerous Ser/Thr kinases, including the AGC, CAMK, and CMGC group of kinases (90). Thr686 is the corresponding residue in Mps1 and, unlike many other Ser/Thr kinases, is autophosphorylated both *in vitro* and *in vivo* (33, 79, 80, 84, 85, 87, 88). Mutation of Thr686 reduces the kinase activity by at least 40-fold *in vitro* and inactivates Mps1 function *in vivo* (33). Phosphorylated Thr686 is likely a feature of active Mps1 kinase as a phospho-T686 antibody can deplete the active kinase (80, 87). What is unique about Thr686 phosphorylation in Mps1 is that the hydroxyl

groups of this residue in other Ser/Thr kinases (e.g., Cdk2) form hydrogen bonds with conserved catalytic residues of the active kinases. The equivalent residue in the P+1 loop of tyrosine kinases is a proline, which is involved in substrate binding. Phosphorylation of the P+1 loop is unique to Mps1, and it is tempting to speculate that modulating the P+1 loop conformation via phosphorylation could be a novel mechanism for kinase activation and that this difference in the P+1 loop is associated with the dual specificity of Mps1 (80).

3.3. Diversity in Phosphorylation Site Selection and Substrate Recognition

Many Mps1 substrates have been identified. Surveying a variety of Mps1 auto- and transphosphorylation sites makes clear that there is no strict consensus phosphorylation sequence associated with the Mps1 kinase. A recent study reported a preference for D/E/N/Q at the -2 position (88), a recognition feature that is also associated with Plk1. This finding suggests the interesting possibilities that Mps1 and Plk1 may share some common physiological substrates and that some of the Mps1 *in vitro* autophosphorylation sites could be targeted by Plk1 *in vivo* (88). Despite these notable preferences, the sites targeted by Mps1 are highly diverse, and it is impossible to predict the authentic Mps1 phosphorylation sites *in vitro* and *in vivo*.

How Mps1 recognizes diverse substrates remains a mystery, although there are some hints that there may be different requirements for Mps1 autophosphorylation and transphosphorylation. As mentioned above, Thr676 mutation affects transphosphorylation more than autophosphorylation (33). Another unexpected finding came from deletion analysis of the C-terminal region of MPS1, a 65-amino-acid region that is disordered in all known Mps1 structures. This region is susceptible to proteolysis, which may explain the disagreement in abundance measurements using N-terminal or C-terminal antibodies (89). Removal of the 65-amino acid (noncatalytic) tail reduces Mps1 transphosphorylation by about sixfold but has little impact on Mps1 autophosphorylation (89). The most straightforward interpretation of this result is that this region of Mps1 is involved in exogenous substrate recognition. The significance of this region is underscored by the fact that, without it, Mps1 is defective in the spindle assembly checkpoint response, demonstrating that the presence of a kinase domain alone is insufficient for Mps1 function *in vivo* (89).

3.4. Dimerization

Kinase autophosphorylation can occur through intermolecular or intramolecular mechanisms. Mps1 transphosphorylation was first shown *in vitro* (33, 80, 84). Induced dimerization of Mps1 is sufficient to activate its kinase activity in cells (84). Mps1 dimerization and transphosphorylation have also been demonstrated using differentially tagged Mps1 constructs in a coimmunoprecipitation assay (39). Finally, kinetic studies of Mps1 phosphorylation *in vitro* support the notion that Mps1 undergoes intermolecular autophosphorylation *in vitro*, as the rate of autophosphorylation increases with increasing concentrations of Mps1 (89). Dimerization of Mps1 may have implications about where Mps1 is initially activated during cell-cycle progression. One proposal is that the kinetochore localization of Mps1 could raise its local concentration, leading to its activation during mitosis via more efficient intermolecular autophosphorylation (84, 91, 92). Although

this may be the case for elevated Mps1 activity during spindle assembly checkpoint signaling, Mps1 activity also needs to increase prior to its relocalization to kinetochores to control centrosome duplication. It is equally possible that the initial activation of Mps1 occurs at the centrosome, where Mps1 is also highly concentrated prior to mitotic entry (Figure 1).

3.5. Mutant and Analog-Sensitive Alleles

Several mutant *MPS1* alleles have been discovered in yeast, fly, and zebrafish. The original yeast temperature-sensitive-for-growth *mps1-1* allele (C696Y/αH/domain XI), as well as five additional temperature-sensitive alleles all arose from point mutations in the kinase domain (7). The mutations varied in their effect on in vitro kinase activity from very severe (e.g., *mps1-1*) to rather mild (e.g., *mps1-6* C642Y/αF/domain IX). The importance of kinase activity for Mps1 function is reinforced by the finding that the hypomorphic nightcap mutation in zebrafish is an Ile843Lys mutation in subdomain VI of the kinase domain, which is conserved in Mps1 kinases as a hydrophobic Ile or Leu residue (655 in hMps1/β6/catalytic loop/domain VI) (54). Finally, a null allele of the *Drosophila* Mps1 ortholog, *ald*^{C3}, was found to contain a nine-amino acid deletion in the kinase domain (codon 369–377 between the β3 and αC/domain III) (25).

The N-terminal, noncatalytic region of Mps1 kinases is also mutated in some alleles of *MPS1* genes. The original hypomorphic *Ald* mutation in *Drosophila*, *ald*¹, is an Arg to His substitution at amino acid 7. Similarly, hypomorphic alleles of yeast *MPS1*, *pac8-1* and *pac8-2*, identified as synthetically lethal with the deletion allele of the kinesin Cin8 (93), are point mutations in the N terminus (M. Winey, unpublished observation). These alleles are likely defective in a kinetochore function of Mps1, as is *mps1-7*, which contains a point mutation in the N terminus and is defective in the spindle checkpoint but competent for SPB duplication (94). The *mps1-8* allele is a temperature-sensitive allele that contains several mutations in the N terminus, which do not affect the kinase activity but are defective in SPB duplication (15).

Analog-sensitive alleles have been a valuable tool in probing kinase function and identifying authentic kinase substrates. The gatekeeper residue of Mps1 is a Met at positions 516 and 602 in yeast and human enzymes, respectively. The yeast Mps1-as1 allele was first created by changing the bulky gatekeeper residue Met to a smaller Gly, which makes the kinase specifically sensitive to a cell-permeable ATP analog inhibitor, 1-NM-PP1 (53). The Mps1-as1 allele not only reaffirmed the function of Mps1 in SPB duplication and the spindle checkpoint but was also used to show that Mps1 acts in bipolar chromosome attachment (38, 53) and to show that Mps1 must be inactivated to exit the cell cycle (70). Mps1-as alleles have also been used to resolve some of the controversies surrounding Mad1 kinetochore recruitment and Aurora B activation by Mps1, which is discussed below (23, 43, 95).

3.6. Small-Molecule Mps1 Inhibitors

The Mps1 kinase has emerged as a novel drug target for cancer therapy. Cincreasin was the first reported small inhibitor of Mps1, though it is not particularly potent with 50% inhibitory concentration (IC₅₀) = 700 μM (96). SP600125, a JNK inhibitor, was found to

inhibit Mps1 off target (97). In recent years, a variety of structurally diverse Mps1 inhibitors have been described (Tables 1 and 2). Several Mps1 kinase catalytic domain crystal structures, apo or complexed with an inhibitor, were solved recently (79, 81–83). These structures helped shed light on how a small-molecule inhibitor binds with the Mps1 kinase and on how to design selective Mps1 inhibitors. Shown in Figure 2*b* is the crystal structure of the Mps1 kinase domain in complex with Mps1-IN-1 to illustrate kinase inhibitor-binding modes (81). As shown in Figure 2, the pyrrolopyrimidine scaffold forms the anchor of the inhibitor. It sits in the adenine-binding pocket, making hydrogen bond interactions between the substitutions on the scaffold and the protein inside the adenosine-binding pocket; these substitutions could also extend out into the solvent-exposed region and phosphate-binding region (Figure 2*c*). Cys604, a hinge residue that varies between kinases, has been explored in designing more selective Mps1 inhibitors (82).

Because Mps1 is an essential gene in the pathogenic fungi *Candida albicans* and significant sequence divergence exists between human and *Candida* Mps1, species-specific inhibitors of Mps1 kinase could be employed as antifungal agents. In an effort to identify novel antifungal chemotherapeutics, the guanylate cyclase inhibitor LY83583 was found to inhibit *Candida* Mps1 without affecting hMps1 activity (98). Further advancing the feasibility of species-specific targeting of Mps1, SP600125, which inactivates human Mps1, has only modest inhibitory effects on *Candida* Mps1 and is nontoxic to *Candida* (98). Thus, sequence variations in Mps1 may offer a window of opportunity for new therapeutics in combating human pathogens.

4. REGULATION OF MPS1 KINASES

To accomplish a myriad of functions, MPS1 kinases must be exquisitely regulated. Indeed, experimental changes in the expression levels of MPS1 kinases (active and inactive) are detrimental in a variety of cell types. In general, MPS1 kinases are expressed at low levels, and the most important regulatory mechanisms operate via the posttranslational mechanisms of phosphorylation (discussed above with reference to the catalytic domain structure) and degradation.

4.1. Transcription

Many mammalian proteins that function in mitosis and mitotic checkpoint signaling, including Mps1, are controlled at the transcriptional level by the E2F family of transcription factors (99–103). Mps1 mRNA peaks in mitosis (11), and E2F4 and, to a lesser degree, E2F1 bind the *MPS1* promoter region (103). In mouse embryonic fibroblasts lacking p107 and p130, which are two Retinoblastoma family transcriptional repressors of E2F transcription, *MPS1* transcription is derepressed, and the mRNA transcribed at a higher level than in the wild-type control. These data suggest that the retinoblastoma E2F complex may be directly involved in repressing *MPS1* transcription in interphase cells (103). Whether *MPS1* transcription is directly regulated by E2F4 family transcription factors remains to be investigated. Finally, *MPS1* mRNA levels are elevated in freshly isolated peripheral blood lymphocyte or T cell blasts (104). IL-2 incubation also induces Mps1 expression in proliferating peripheral blood lymphocyte blasts (104). Thus, transcription of Mps1 is upregulated when cells enter the cell cycle and transit through mitosis.

4.2. Localization

Subcellular localization of Mps1 is both spatially and temporally regulated during cell-cycle progression (29, 30, 66, 86). In mammalian cells, Mps1 primarily resides within the cytosol during G1. In late G2, Mps1 accumulates on centrosomes and the nuclear envelope (29, 66, 86, 105). At the G2/M boundary, Mps1 abruptly enters into the nucleus prior to nuclear membrane breakdown (106). Nuclear import of Mps1 requires two LXXLL motifs in the N terminus of Mps1. In interphase cells, Mps1 likely shuttles between nucleus and cytosol constantly, as leptomycin B treatment can lead to redistribution of Mps1 into the nucleus (106). As cells move into prophase, Mps1 preferentially associates with kinetochores and is slowly lost until the onset of anaphase, when Mps1 disassociates from kinetochores (91, 92, 105). The noncatalytic N-terminal domain is necessary and sufficient for localization to kinetochores in isolation, whereas the C-terminal domain by itself cannot locate hMps1 to kinetochores (23, 29, 86, 107). However, the function of the kinetochore-targeting signal in the N terminus could be masked by the sequence in the C-terminal region of Mps1. For example, without phosphorylation of Ser844 of XIMps1 by MAPK, XIMps1 cannot relocate to kinetochores even though the N-terminal-targeting signal is intact (108). Similar observations were made with hMps1 (86, 109). These results imply that the C-terminal region of Mps1 may regulate access to the kinetochore-targeting signal that resides in the N terminus of Mps1.

Besides MAPK, two other kinases, PRP4 (pre-messenger RNA processing 4) and Aurora B, have been implicated in the regulation of Mps1 kinetochore localization. PRP4 protein kinase associates with kinetochores during mitosis and is required for efficient Mps1 kinetochore targeting (110). Depletion of PRP4 induces mitotic acceleration, chromosome misalignment, and defects in Mad2 localization, which are phenotypes observed with inactivation of Mps1. The mechanism by which PRP4 regulates Mps1 remains to be determined. Similarly, as mentioned above, inhibition of Aurora B by various methods reduces Mps1 localization to unattached kinetochores throughout mitosis (46). However, Mad2 recruitment to kinetochores, which requires Mps1 activity, is significantly affected only in early mitosis, suggesting that Aurora B may regulate the timing or amplitude of Mps1 activation. Delayed Mps1 activation caused by Aurora B inhibition also causes a delay in establishment of the spindle checkpoint. The defects in Mps1 kinetochore targeting in early mitosis and spindle checkpoint delay can be rescued by tethering Mps1 to the kinetochore (46). This result suggests that Aurora B acts upstream in promoting early mitotic Mps1 kinetochore targeting. The effects of Aurora B on Mps1 have also been investigated in conjunction with Hec1/Ndc80, a core component of the kinetochore essential for organizing microtubule attachment sites (51). Hec1 is required for the recruitment of Mps1 kinase and Mad1/Mad2 complexes to kinetochores (111). Although there has been no report of direct interaction between Hec1 and Mps1, the budding yeast Hec1 ortholog, Ndc80, directly interacts with yeast Mps1 (50). Hec1 may well be the kinetochore-bound acceptor for Mps1 in mammalian cells. Consistent with a direct role of Hec1 on Mps1 targeting, depletion of Hec1 results in more dramatic effects on Mps1 targeting than Aurora B inactivation. However, one intriguing possibility is that Aurora B may act by phosphorylating the N terminus of Hec1 to regulate Mps1 kinetochore localization (51). It

will be interesting to investigate whether Hec1 phosphorylation by Aurora B creates a docking site for Mps1 to bind kinetochores.

Mps1 centrosome localization is mediated by its N-terminal domain, but the precise motifs have not been characterized. Nonetheless, distinct regions of the N terminus of the yeast and human enzymes have been implicated in its centrosome function. In yeast, deletion analysis with the clever use of analog-sensitive alleles revealed that amino acids 201–300 are required for SPB duplication and are distinct from amino acids 151–200, which are required for chromosome biorientation (14). Similarly, a region internal to the N terminus of hMps1, amino acids 420–507, called the MDS (Mps1 degradation signal), is critical in controlling centrosomal levels of hMps1 (112). Deletion of this region stabilizes the protein and localizes it to centrosomes, driving excess centrosome production. The MDS is recognized by centrosome-localized OAZ (antizyme), which is responsible for the degradation of centrosomal Mps1 (113). The level of the centrosomal Mps1 is also regulated by its phosphorylation on Thr468 within the MDS by Cdk2 (112), which stabilizes the protein, opposing OAZ-mediated degradation to create a regulatory circuit that controls centrosomal hMps1 levels (22).

Phosphorylation of Mps1 is also important for its localization. Mutating the nine autophosphorylation sites in the N terminus of Mps1 causes a significant decrease in kinetochore targeting of Mps1 without affecting centrosomal localization in SW480 cells (86). This result suggests that the kinetochore-targeting signal is independent of the centrosome-localization signal. Among these sites, T12 and S15 appear to be critical in mediating Mps1 accumulation on kinetochores (86). Consistent with these results, kinase-inactive Mps1, expressed in SW480 cells, exhibits reduced kinetochore relocation upon depletion of endogenous Mps1 (86). Reduced kinetochore localization of endogenous Mps1 was also observed when US2OS cells were treated with the inhibitor NMS-P715 (82). However, kinetochore accumulation of transiently transfected Mps1 or Mps1 KD in HeLa increases when cells are treated with the inhibitor AZ3146, suggesting that kinase activity inhibits kinetochore recruitment in HeLa cells (39). It is interesting to note that the results of AZ3146 on recruitment of spindle checkpoint proteins to kinetochores depend on whether the inhibitor is administered before or after mitotic entry. To reconcile the timing effect, it was proposed that there are two phases of checkpoint protein recruitment to kinetochores: an initial phase prior to mitotic entry and a subsequent maintenance phase during mitosis (39). Increased Mps1 accumulation on kinetochores in the presence of AZ3146 may be a result of reduced release from kinetochores. In this way, the Mps1 kinase activity may be required for targeting to kinetochores and also for release from kinetochores. The details about these requirements remain unsettled and may, like the role of Mps1 at the centrosome, depend on the cell type and experimental conditions.

4.3. Degradation and Inactivation

The major route of Mps1 inactivation is degradation. The expression and activity of Mps1 are cell cycle dependent in both yeast and mammalian cells (70, 89, 107). Expression peaks in metaphase and declines when cells enter anaphase. Timely inactivation of Mps1 is required for proper cell-cycle progression and termination of spindle checkpoint signaling.

During normal cell-cycle progression, Mps1 is partially degraded in anaphase by the ubiquitin E3 ligase APCCdc20. Overexpression of Mps1 in anaphase can activate the checkpoint by inhibiting APCCdc20 and blocks mitotic exit in yeast (70). There are three D-boxes in the N terminus of yeast Mps1, which are required for proteolysis by APCCdc20 (70). Therefore, APCCdc20 and Mps1 are mutually inhibitory, forming a double negative feedback loop. This circuit may enable the metaphase to anaphase transition to be switch like and irreversible. Human Mps1 contains only one canonical D-box, and it is sequentially degraded by APCCdc20 and APCCdh1 in a D-box-dependent manner (114). A D-box-deficient hMps1 perturbs normal mitosis and causes centrosome overreplication in human cells. Efficient degradation of Mps1 is also aided by Ufd2, a U-box-containing ubiquitin-protein ligase, in both yeast and mammalian cells (115). Hence, proteolysis regulates temporal expression and activity of Mps1.

Degradation of Mps1 also occurs spatially. Centrosome accumulation of hMps1 is greatly enhanced by phosphorylation at Thr468 by Cdk2 (112). Phosphomimetic mutations at Thr468 or deletion of the region surrounding Thr468 protects Mps1 from degradation at centrosomes (112). Yeast Mps1 is stabilized by CDK phosphorylation of Thr29, but the mechanism is unknown (20). Kinetochore-associated Mps1 also may be regulated by proteolysis. The retention time for Mps1 on unattached kinetochores in checkpoint-arrested cells is about 10 s (91). Treatment with both MG132 and Mps1 inhibitors enhances its accumulation at kinetochores (39), suggesting a role for proteolysis. Because Mps1 has a distinct subcellular localization during cell-cycle progression, it is possible that different pools of Mps1 are differentially regulated by proteolysis.

Another possible mechanism of Mps1 inactivation is dephosphorylation. Mps1 is hyperphosphorylated in mitosis (29, 30) and rapidly dephosphorylated upon anaphase entry (29). To date, phosphatases that specifically act on MPS1 family kinases have not been identified. Early in vitro studies show that PTP1B can remove the phospho-Tyr epitope produced by mouse Mps1 autophosphorylation (10). PP1 γ has also been shown to dephosphorylate Mps1 in vitro (77). Whether any of these phosphatases inactivate Mps1 in vivo remains unknown.

4.4. Misregulation in Tumor Cells

Like many cell-cycle regulators, Mps1 transcription is deregulated in a variety of human tumors. Elevated Mps1 mRNA levels are found in several human cancers, including thyroid papillary carcinoma, breast cancer, gastric cancer tissue, bronchogenic carcinoma, and lung cancers (8, 116–120). Furthermore, high levels of Mps1 correlate with a high histological grade in breast cancers (119). Conversely, Mps1 mRNA is markedly reduced or absent in resting cells and in tissues with a low proliferative index (11). Thus, there is a correlation between elevated Mps1 levels and cell proliferation as well as with tumor aggressiveness. Consistent with the notion that oncogenic signaling promotes Mps1 expression, the levels and activity of Mps1 are increased by 3- and 10-fold, respectively, in human melanoma cell lines containing the B-Raf^{V600E} mutant (121). Inhibition of B-Raf or MEK1 reduces Mps1 expression (109, 121).

The observation that tumor cells frequently overexpress spindle checkpoint proteins is perplexing as the conventional wisdom would postulate that tumor cells would have a weakened checkpoint, contributing to chromosome missegregation and aneuploidy. Indeed, significant evidence from yeast to mice supports the notion that a weakened checkpoint leads to chromosome instability (122). However, mutations in key checkpoint proteins are rare in human tumors, and correlative evidence showing that compromised checkpoint signaling directly contributes to the development of human tumors has been elusive. *MPS1* missense mutations have been found in the noncatalytic N terminus in bladder (123) and lung cancers (124), as well as in the kinase domain in pancreatic (125) and lung cancers (124). Interestingly, frameshift mutations that truncate the protein arise from microsatellite instability in the hMps1 gene in gastric (126) and colorectal cancers (127). Thus, mutations in hMPS1 have been detected in tumor-derived cells; however, their influence on tumorigenesis is not known.

The prevalence of high levels of checkpoint protein expression, such as Mps1, in human tumors prompts an alternative hypothesis regarding the potential role of checkpoint proteins in cancer cells, i.e., overexpression of these proteins may promote either cancer initiation or survival of aneuploid cancer cells (119, 128). Accordingly, reductions in key checkpoint proteins should severely decrease human cancer cell viability. This prediction is confirmed for several checkpoint proteins, including Mps1 (66, 119), BubRI (129), and Mad2 (130, 131). Suppression of Mps1 expression in Hs578T breast cancer cells also reduces the tumorigenicity of these cells in xenografts. Cancer cell death is likely the result of severe chromosome segregation errors when the checkpoint is disabled. Interestingly, cells that survived reduced Mps1 levels often display lower levels of aneuploidy, suggesting that lower levels of Mps1 potentially inactivating the checkpoint are incompatible with aneuploidy (119). This concept is in excellent agreement with the observation that reduction in checkpoint proteins makes tumor cells more sensitive than untransformed human fibroblasts to low doses of spindle poisons (129). Differential cellular responses to checkpoint inhibition between normal and tumor cells could be key in developing new anticancer drugs targeting hMps1. Recent results from at least one hMps1 inhibitor, NMS-P715, show great promise in preclinical cancer models (82). We anxiously await the determination of whether inhibitors of Mps1 are efficacious and safe, either as single agents or in combination, in clinically relevant settings.

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Glossary

MPS1	monopolar spindle 1
SPB	spindle pole body

Centrosome	the cellular structure that contains centrioles, nucleates microtubule formation, and organizes mitotic spindles
Autophosphorylation	the action of a kinase adding one or more phosphate groups to itself
Dual-specificity protein kinase	a protein kinase that exhibits Ser/Thr and Tyr phosphorylation activities
Spindle checkpoint	this mechanism ensures proper chromosome attachment to microtubules prior to chromosome segregation (aka spindle assembly checkpoint or mitotic checkpoint)
Kinetochores	this structure, assembled at centromeres, captures spindle microtubules and serves as the signaling platform for the spindle checkpoint
APC	anaphase-promoting complex
Syntelic attachments	an aberrant chromosome attachment, where both sister chromosomes are attached to a single spindle pole instead of a bipolar attachment
Ald	<i>Drosophila</i> Mps1 homolog
Transphosphorylation	the action of a kinase mediating transfer of phosphate to its cognate substrates
Analog-sensitive allele	a variant protein kinase carrying a mutation at the gatekeeper residue, allowing it to accept a bulky analog of ATP for inhibition
Gatekeeper residue	the residue located in the ATP-binding pocket of a protein kinase that controls the selectivity and sensitivity to small-molecule inhibitors

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SUMMARY POINTS

1. Mps1 kinases with their conserved, C-terminal kinase domains are widely, but not ubiquitously, distributed among eukaryotes.
2. Mps1 kinases are localized at kinetochores, where they function with Aurora B kinases to ensure proper bipolar attachment.
3. Mps1 kinases act at an early step in the spindle checkpoint, and the functions of most of the checkpoint proteins are dependent, directly or indirectly, on Mps1 activity.
4. Mps1 kinases are found in centrosomes, are required for SPB (centrosome) assembly in yeast, and influence centrosome assembly in mammals.
5. Mps1 kinases exhibit significant levels of autophosphorylation, which is essential for its activation and subcellular localization.
6. Mps1 kinases are inactivated by APC-dependent degradation, which is necessary for cells to exit mitosis correctly.
7. Mps1 kinase genes are misregulated in tumors, supporting the hypothesis that the checkpoint is necessary for the viability of aneuploid tumor cells.
8. Mps1 kinases have become of interest for the development of small-molecule inhibitors. It is anticipated that some of the inhibitors discovered will be tested in clinical trials.

FUTURE ISSUES

Since the discovery of the first Mps1 allele, there has been tremendous progress in understanding the biological function and underlying mechanisms of this protein kinase. However, many important questions regarding Mps1 function remain. For example:

1. What are the molecular mechanisms of Mps1 in its known functions in kinetochore attachment, the spindle checkpoint, and centrosome assembly? Particularly, what are the pertinent Mps1 substrates for these various functions?
2. What are all of the Mps1 kinase functions? Mps1 kinases function in genotoxic stress, the actin cytoskeleton, and likely in other contexts that remain to be identified.
3. Is the lack of Mps1 paralogs functionally significant? Could the myriad and complex functions of these kinases require a single isoform for correct regulation?
4. What protein kinases carry out the various functions of Mps1 kinases in organisms lacking this kinase?
5. What governs Mps1 subcellular localization and its changes during the cell cycle?
6. Are Mps1 kinases inactivated by reversible mechanisms, such as dephosphorylation? A biosensor assay for active Mps1 would be critical for this work, and it would be useful in examining Mps1 at its various cellular locations.
7. What is the active conformation of Mps1, and what can it tell us about the mechanisms of Mps1 activation and substrate recognition?
8. Will Mps1 be found to be a good drug target for antitumor therapy?

Answers to these questions will undoubtedly provide a more lucid and exciting picture of how Mps1 orchestrates normal cell-cycle progression and its deviation in tumorigenesis.

Figure 1.

Localization of Mps1 in vertebrate mitotic and interphase cells. (a) Kinetochores localization of yellow fluorescent protein (YFP) Mps1 in mitotic SW480 cells. Anti-centromere antibodies (ACA) and 4',6-diamidino-2-phenylindole (DAPI) were used to stain kinetochores and chromosomes. (b) YFP Mps1 is localized to centrosomes and the cytosol during interphase. Centrosomes and nuclei were stained by anti- γ -tubulin and DAPI, respectively.

Figure 2.

(a) Ribbon representation of the structure of the Mps1 catalytic domain. Key structural elements are labeled. The structure has been rendered from the Protein Data Bank (PDB) entry 3DBQ, using the Maestro interface from Schrödinger. The dotted lines represent the disordered regions in the activation loop, the loop between αEF and αF and also at the C-terminal tail. (b) Ribbon representation of the structure of Mps1 in complex with a small-molecule inhibitor, Mps1-IN-1. The structure has been rendered from the PDB entry 3GFW using the Maestro interface. The residues in the hinge region are shown. (c) Illustration of the inhibitor-binding mode. The gatekeeper residue M602, hinge region residues that interact with the inhibitor and the ATP-binding pocket are shown. The dotted lines represent hydrogen bonds.

Figure 3. Multiple sequence alignment of the kinase domain of Mps1 from representative species using PRALINE multiple sequence alignment (<http://www.ibi.vu.nl/programs/pralinewww/>). Amino acid conservation is shown by a color-coded heat map from unconserved to conserved. The secondary structure of Mps1 is shown above the Mps1. Key amino acid residues are shown below the alignment.

Table 1

Summary of published potent hMps1 inhibitors

Inhibitor	Structure	IC ₅₀ (nM)	References (patent number)	Protein Data Bank crystal structure
SP600125		692	78, 83, 97	2ZMC
AZ3146		35	39 (WO2009024824)	
Mps1-IN-1		370	81 (WO2009032694)	3GFW
Mps1-IN-2		145	81 (WO2010080712)	3H9F
NMS-P715		8	82 (WO2009156315)	2X9E
MPI-0479605		3.5	106, 132	
Reversine		3/6	40	

Inhibitor	Structure	IC ₅₀ (nM)	References (patent number)	Protein Data Bank crystal structure
Staurosporine		102	83	3HMO
Cpd4		38,000	83	3HMP

Table 2

Additional patented hMps1 inhibitors

Claimed structure	Example	IC ₅₀ (nM)	Patent number (company)
3	JP2010111624 (Shionogi & Co., Ltd.)
2.6	WO2010124826 (Bayer Pharma)
1	WO2011063907 WO2011063908 WO2011064328 (Bayer Pharma)
3.9	WO2011013729 (OncoTherapy Science, Inc.)
4	WO2011016472 (OncoTherapy Science, Inc.)