

Centrosomes and checkpoints: the MPS1 family of kinases

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Introduction

Mitotic progression is controlled by a number of protein kinases in addition to the global cell cycle control exhibited by cyclin dependent kinases (CDKs). These include members of the Polo, Bub1, NimA, Aurora and MPS1 families of kinases that have various roles in the assembly, function and disassembly of the mitotic spindle (Nigg, 2001). Interestingly, many also have roles in the checkpoints that monitor mitotic progression. Here we review the work on the MPS1 family of kinases.

MPS1 family of kinases

The MPS1 (*MonoPolar Spindle 1*) family was first described in budding yeast based on its mutant phenotype (Winey *et al.*, 1991). A monopolar spindle is a consequence of failure in the duplication of the yeast centrosome, called the spindle pole body (SPB). The SPBs nucleate the formation of all of the microtubules in the yeast cell and act as the spindle poles. As such, the SPB, like the centrosome in other organisms, must be duplicated once and only once per cell cycle (reviewed in Adams and Kilmartin, 2000). A screen for mutations in this process led to the identification of the *mps1-1* allele (Winey *et al.*, 1991). The *MPS1* gene was shown to be essential and to encode a protein kinase homolog (Lauze' *et al.*, 1995; Poch *et al.*, 1994). Mps1p is a dual specificity protein kinase *in vitro*, capable of autophosphorylation on serine, threonine and tyrosine residues (Lauze' *et al.*, 1995). However, activity towards an exogenous substrate, myelin basic protein, revealed phosphorylation only on serine and threonine residues (Lauze' *et al.*, 1995). Recently, Mps1p has been shown to phosphorylate tyrosine *in vitro* on an exogenous poly (Tyr-Glu) peptide (Zhu *et al.*, 2000). The ability of

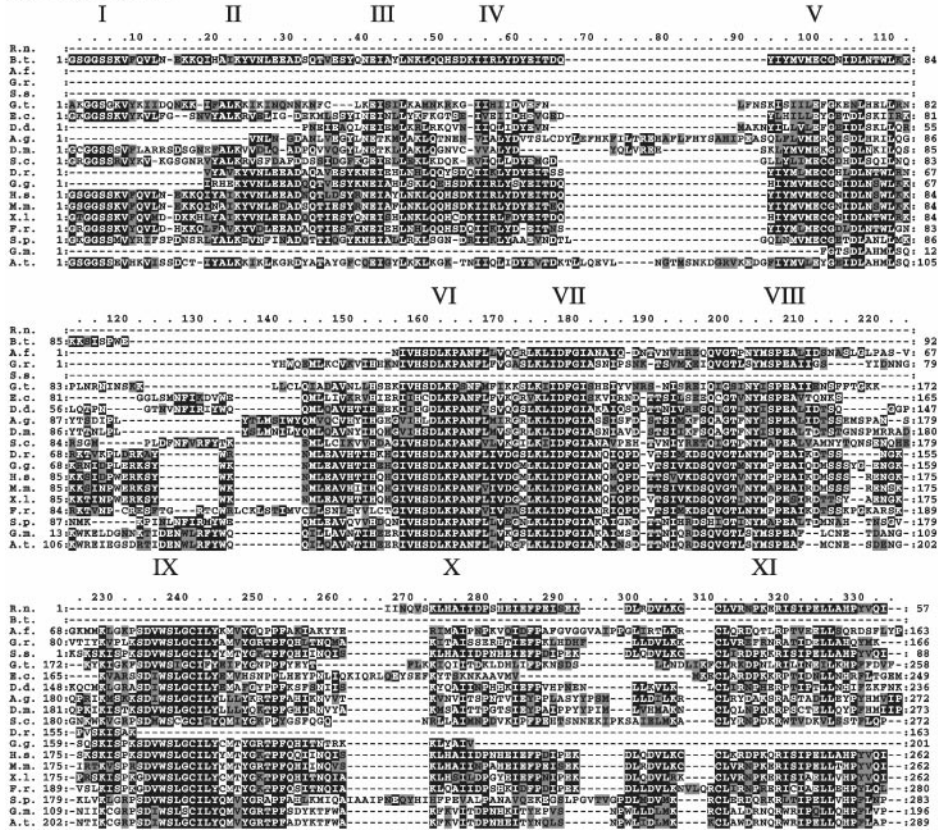
protein kinases from this family to autophosphorylate on tyrosine *in vitro* lead to the serendipitous identification of vertebrate members of the family. Library screening with anti-phosphotyrosine antibodies identified the human (pyt/tyk, Lindberg *et al.*, 1993; Mills *et al.*, 1992) and the mouse (esk, Douville *et al.*, 1992) homologs. Both pyt/tyk and esk were shown to be protein kinases *in vitro* and the mouse enzyme (esk) was shown to phosphorylate tyrosines on an exogenous substrate *in vitro* (Douville *et al.*, 1992). The *Xenopus* gene was identified from a degenerate PCR screen and has been shown to have protein kinase activity (Abrieu *et al.*, 2001). The *S. pombe* gene, mph1, was discovered in a genetic screen, but its kinase activity has not been directly tested (He *et al.*, 1997). Other likely family members have been identified by searching either genome (i.e., *Drosophila*) or EST databases (see Figure 1). Curiously, while members of the MPS1 family of protein kinases are widely distributed in the eukaryotic world, an unambiguous homolog is absent, at least at this point, in the *C. elegans* genome, although another worm, *Globodera rostochiensis*, does have an EST for the gene (see Figure 1).

Sequence alignment of the C-terminal kinase domains found in MPS1 family members is shown in Figure 1. The similarity among the family as a whole is limited to the kinase domain wherein the amino acid identity is at least 33% between any two members, and is as high as 90% between mouse and human. Particularly striking in the alignment is the conservation in subdomain VI in which 11 amino acids are completely conserved. The overall organization of the proteins are similar in that they each have an N-terminal extension of up to 550 amino acids and a much shorter C-terminal extension. Within these amino and carboxyl terminal extensions no detectable motifs exist, and no apparent homology is observed between vertebrates and invertebrates. Nonetheless, these regions are quite similar when compared between closely related species (i.e., mouse and human, see Figure 1).

Beyond the demonstration that these enzymes are protein kinases, it is of interest to determine their substrate recognition site, and to determine if these proteins have any other activities. Few substrates of these kinases have been identified, as will be discussed below, and only one of the budding yeast substrates, the SPB component Spc110p, has been analysed in detail (Friedman *et al.*, 2001). Mps1p was shown to phosphorylate Spc110p on a serine and on two

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A.
SUBDOMAINS:



B.

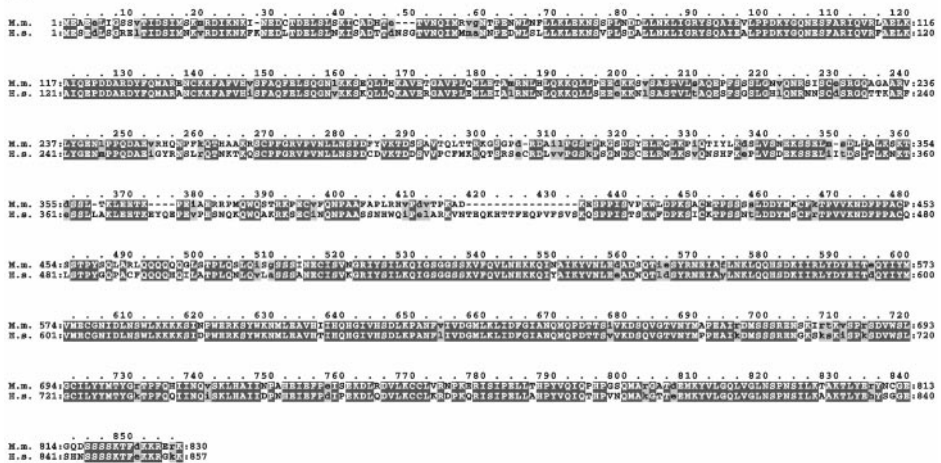


Figure 1 Alignment of MPS1 family members. (a) Alignments of kinase domains for MPS1 family members. Kinase subdomains are labeled I-XI (Shutz and Winey, 1998). Abbreviations and accession numbers for family members: A.f. *Aspergillus fumigatus* (gnl|TIGR_5085|508), A.g. *Anopheles gambiae* (predicted from genome scaffold; AAAB00000000), A.t. *Arabidopsis thaliana* (flowering plant) (CAA72680), B.t. *Bos taurus* (cow) (BM258940), D.d. *Drosophila melanogaster* (fruit fly) (AAF55450), D.r. *Danio rio* (Zebra fish) (BM316342), E.c. *Encephalitozoon cuniculi* (NP_584578), F.r. *Fugu rubripes* (Predicted from genome scaffold), G.g. *Galus galus* (chicken) (AL586909), G.m. *Glycine max* (soybean) (BE329881), G.r. *Globodera rostochiensis* (nematode) (BM343738), G.t. *Giardia theta* (giardia) (NP_113182), H.s. *Homo sapiens* (NP_003309), M.m. *Mus musculus* (common mouse) (B44439), R.n. *Rattus norvegicus* (Norwegian rat) (BQ203389), S.c. *Saccharomyces cerevisiae* (AAB34233), S.p. *Schizosaccharomyces pombe* (O94235), S.s. *Sus scrofa* (pig) (AW416739), X.l. *Xenopus laevis* (frog) (AAK27843). Genome databases can be found at: <http://www.ncbi.nlm.nih.gov/BLAST/> <http://www.ncbi.nlm.nih.gov/BLAST/fugu.html>; <http://www.ncbi.nlm.nih.gov/genome/seq/DrBlast.html>; <http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/agambiae.html>; http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/genom_table.cgi?organism=euk; Fugu Home page (DOE Joint Genome Institute). (b) Alignment of full length proteins from mouse (M.m.) and human (H.s.)

threonine residues; unfortunately, sequence comparison of these three sites does not suggest a consensus phosphorylation sequence for Mps1p (Friedman *et al.*, 2001). As mentioned above, the non-catalytic domains of the proteins do not suggest other possible functions, but a recent high-through-put screen of almost all (93.5%) of the yeast proteins showed that Mps1p could bind phospholipids. This result was rather unexpected although a number of other protein kinases were also reported to have this activity (Zhu *et al.*, 2001). The binding of Mps1p to phospholipids has not been confirmed *in vivo* and it is unclear whether this binding suggests another activity independent of protein phosphorylation.

Mps1p in spindle pole duplication

As previously mentioned, yeast Mps1p was originally identified through its role in SPB (the yeast centrosome) duplication (Winey *et al.*, 1991). The duplication of the SPB in G1 is a critical cell cycle event necessary for the formation of a normal bipolar spindle (reviewed in Adams and Kilmartin, 2000). The original mutation, *mps1-1*, causes cells to fail in SPB duplication at the restrictive temperature resulting in mitotic cells with a single SPB. (Winey *et al.*, 1991). These cells replicate their DNA and grow a bud, suggesting that Mps1p is not a general regulator of cell cycle progression, but specifically regulates SPB duplication. Furthermore, the unduplicated SPB observed by electron microscopy (EM) in these cells has a unique morphology suggesting, in combination with execution point and epistasis experiments, that Mps1p is required for a medial step in SPB duplication (Winey *et al.*, 1991). Finally, Mps1p recently has been reported to localize to SPBs, suggesting that it may act at SPBs to control their assembly (Castillo *et al.*, 2002). Interestingly, unlike other mutants that fail in SPB duplication and form a monopolar spindle, *mps1-1* cells do not arrest in mitosis but instead go on to inappropriately segregate their DNA without a functional spindle (Winey *et al.*, 1991). This observation led to the demonstration of an additional role for Mps1p in the spindle assembly checkpoint that will be discussed later (Weiss and Winey, 1996).

Additional mutant alleles of *MPS1* were identified by several groups in screens for mutations in yeast that caused defects in mitotic spindle morphology or spindle function, or by various genetic techniques (reviewed in Schutz and Winey, 1998). All of these mutations in *MPS1*, including the original *mps1-1*, were found to be single amino acid substitutions in the kinase domain, some changing highly conserved residues (Schutz and Winey, 1998). In general, strains containing any of these new alleles of *MPS1* behaved the same as those containing *mps1-1*, with the notable exception of those with the *mps1-737* allele. At restrictive temperature, *mps1-737* strains fail in SPB duplication, but the terminal morphology of the SPBs is distinct from that observed in strains containing the other *MPS1*

mutations (Figure 2, (Schutz and Winey, 1998)). Indeed, the defect is very similar to what is observed in strains defective in the *MPS2*, *NDC1* or *BBP1* genes, which together define a late step in SPB duplication (Schramm *et al.*, 2000; Winey *et al.*, 1991, 1993). Intragenetic complementation of *MPS1* alleles supported the notion that *MPS1* has multiple roles in SPB duplication (Schutz and Winey, 1998). Recently, new alleles of *MPS1* were created by PCR mutagenesis of the non-catalytic N-terminal domain. The mutagenized collection was screened for alleles defective in only one function of the kinase (SPB duplication or the spindle assembly checkpoint, discussed below). The *mps1-8* allele is a conditional allele identified from this screen that is only defective in SPB duplication (Castillo *et al.*, 2002). The SPB observed at the restrictive temperature in these cells has a short half-bridge, a SPB morphology not seen with other *mps1*-alleles, presumably revealing another requirement of the kinase in SPB duplication. Figure 2 shows the placement, based on SPB morphology, of the *mps1* mutants in SPB duplication. It is clear that Mps1p is required for a number of distinct steps in building a functional SPB. In addition, several of these alleles have been used to show that Mps1p is also required for both rounds of SPB duplication in meiosis, those necessary to form the MI and MII spindles (Straight *et al.*, 2000).

One obvious mechanism by which Mps1p could regulate SPB duplication is by phosphorylation of SPB components. Fortunately, many genes encoding components of the SPB have been identified and many have been shown to encode phosphoproteins (Wigge *et al.*, 1998). Three SPB components have been shown to be Mps1p substrates *in vitro*; Spc98p, Spc110p and Spc42p (Castillo *et al.*, 2002; Friedman *et al.*, 2001; Pereira *et al.*, 1998). Spc98p is a component of the 6S gamma-tubulin complex in yeast that is responsible for nucleation of microtubules (Knop and Schiebel, 1997).

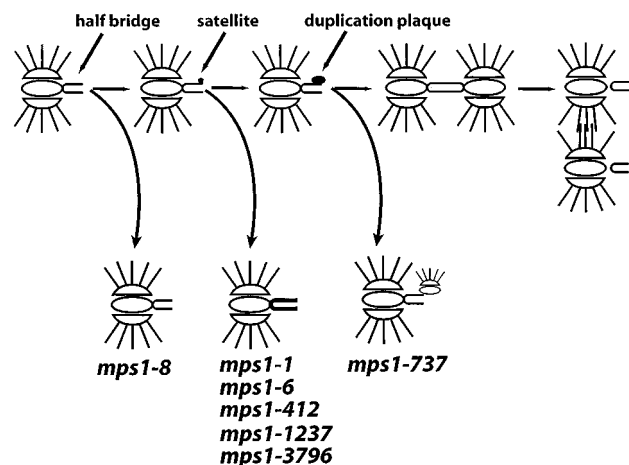


Figure 2 Mps1p acts in multiple steps of SPB duplication. Schematic diagram of SPB duplication in *S. cerevisiae* similar to Adams and Kilmartin (2000). *mps1* mutants are listed with their terminal SPB morphology

Spc110p binds 6S gamma-tubulin complexes at the nuclear side of the SPB (Knop and Schiebel, 1997; Sundberg and Davis, 1997). Spc42p is a component of the core of the SPB (Donaldson and Kilmartin, 1995). Cell cycle profiles of Spc98p and Spc110p show that slower mobility, phosphorylated forms of these proteins accumulate as cells approach mitosis and that Spc98p shows significant levels of phosphorylation in cells arrested in mitosis with nocodazole (Friedman *et al.*, 1995; Pereira *et al.*, 1998). The phosphorylated forms of Spc98p, Spc110p, and Spc42p have been shown to depend on Mps1p activity *in vivo*, and Mps1p and Spc42p were also found to bind each other by co-immunoprecipitation (Castillo *et al.*, 2002). Interestingly, Spc42p overexpression leads to assembly of additional material into the SPB (Donaldson and Kilmartin, 1995). Using this observation as the basis of an *in vivo* assembly assay, it was shown that Mps1p is required for normal Spc42p assembly (Castillo *et al.*, 2002).

Friedman *et al.* (2001) have mapped *in vivo* and *in vitro* phosphorylation sites on Spc110p, identifying a combination of Mps1p and Cdc28p (the budding yeast Cdk kinase) phosphorylation sites. Mutagenesis of these sites gave no phenotype in an otherwise wild-type background, but the alleles were found to be lethal in combination with a mutation in *SPC97*, which encodes another subunit of the gamma-tubulin complex (Friedman *et al.*, 2001; Knop and Schiebel, 1997). It may be that severe phenotypes resulting from loss of Mps1p phosphorylation will not be revealed until the phosphorylation sites on Spc110p, Spc98p and possibly other components are examined in combination. Such a result would suggest that Mps1p-directed phosphorylation of multiple components is required for SPB assembly or function. Other possibilities are that some Mps1p phosphorylation events are not essential or some functions of Mps1p are redundant with other kinases or mechanisms. Overall, the analysis of Mps1p in yeast has shown that this kinase is found at SPBs, is required for multiple steps in SPB duplication/assembly and is likely to function by directly binding and phosphorylating SPB components to direct their proper assembly or to stabilize assembled complexes.

The analysis of Mps1p in yeast begs the question about the function of this conserved family of kinases in other organisms. The mouse enzyme, mMps1p (formerly esk), has been localized to centrosomes throughout the cell cycle, both at endogenous levels and when fused to GFP (Fisk and Winey, 2001). Functional analysis of mMps1p has been performed in S phase arrested NIH3T3 cells, which normally undergo only a single round of centrosome duplication (Fisk and Winey, 2001). Overexpression of mMps1p causes centrosomes to reduplicate in these cells, as confirmed by EM analysis of centriole numbers. Interestingly, overexpression of mMps1-KD (kinase dead) blocks centrosome duplication, again indicating a role for the kinase in centrosome duplication. mMps1p-KD was also shown to block centrosome duplication in other cell types which normally reduplicate centrosomes

during S phase arrest (CHO, U2OS) (Fisk and Winey, 2001). These studies demonstrate that a vertebrate Mps1p kinase is involved in centrosome duplication. Although little is known about which step(s) in duplication is controlled by the kinase or about its centrosomal substrates, the centrosome duplication function of mMps1p requires Cdk2, a major regulator of the process (discussed later).

Human Mps1p (hMps1p, formerly pyt/ttk) has been reported to be at centrosomes in HeLa cells using both antibody staining and GFP-tagged alleles (TG Yen, personal communication). However, a recent report on hMps1p reveals a role for this kinase in the spindle assembly checkpoint (discussed below), but finds no evidence suggesting that the kinase functions in centrosome duplication (Stucke *et al.*, 2002). These studies were carried out in U2OS cells, a human osteosarcoma derived cell line that reduplicates its centrosomes during S phase arrest. The authors did not find hMps1p at the centrosomes and various functional analyses – antibody injections, RNA interference and over-expression of wild-type and kinase dead alleles – failed to find evidence suggesting that the kinase acted in centrosome duplication. Because of the very high sequence conservation between the mouse and human Mps1p, this result seemed unexpected. These seemingly contradictory results are difficult to compare because different reagents and protocols were used in each case. For example, while Stucke *et al.* (2002) reported that overexpression of hMps1p-KD does not prevent the reduplication of centrosomes in U2OS cells, Fisk and Winey (2001) reported that overexpression of mMps1p-KD blocks the initial round of centrosome duplication in these cells, as well as in NIH3T3 and CHO cells. In the Stucke *et al.* (2002) experiment, hMps1p-KD was overexpressed six-fold relative to endogenous levels from a tetracycline regulated promoter and cells were arrested throughout S phase by a single treatment with HU. In Fisk and Winey (2001), mMps1p-KD was overexpressed 50-fold relative to endogenous levels from the SV40 promoter and cells were arrested in early S phase by double thymidine block. The differences between these experiments may result from the different dosages of the respective kinase dead proteins, or from the different types of S phase arrest used (Fisk and Winey, 2001; Stucke *et al.*, 2002). More study is required to determine if Mps1p kinases regulate centrosome duplication in all vertebrates or only in select organisms or cell types.

The possibility that Mps1p kinase is not a ubiquitous regulator of spindle pole body or centrosome duplication is raised by the observation that the *C. elegans* genome lacks a clear Mps1p ortholog. *C. elegans* does have a unique protein kinase, encoded by the *zyg-1* gene (O'Connell *et al.*, 2001) that is required for centrosome duplication and perhaps *zyg-1* carries out a function similar to that of Mps1p in centrosome duplication. Furthermore, the fission yeast *S. pombe* MPS1 ortholog, *mph1*⁺, is clearly involved in the spindle assembly checkpoint (discussed below), but does not appear to have a function in SPB duplication.

In fact, the *mph1⁺* gene is not essential (He *et al.*, 1997), consistent with other genes in the checkpoint pathway and contrary to genes required for spindle pole duplication. It would seem that pole duplication in these organisms uses other kinases or mechanisms to accomplish the job done by Mps1p. Identifying the regulatory mechanisms in these cells that do not use Mps1p to duplicate SPBs will be important to determining how pole duplication is accomplished and may reveal redundant systems controlling duplication that could help address some of the above results in vertebrate cells. Nonetheless, new genes may still emerge from the genome projects for these two organisms, so it is possible that the Mps1p orthologs have yet to be identified.

Mps1p in the spindle assembly checkpoint

The spindle assembly checkpoint monitors proper attachment of chromosomes to the mitotic spindle (reviewed in (Gillett and Sorger, 2001)). Genes in the pathway were first identified by mutations in budding yeast that failed to arrest properly in the presence of the microtubule poisons nocodazole or benomyl (Hoyt *et al.*, 1991; Li and Murray, 1991). Five genes in this pathway were identified (*MAD1*, 2, 3 and *BUB1*, 3), all nonessential, indicating that the pathway is not essential in budding yeast. It was later shown that mutations, such as *cdc31-2*, that give rise to a monopolar spindle trigger the checkpoint because of the defective spindle and arrest in mitosis (Weiss and Winey, 1996). Important to the discussion here is that yeast containing mutations in the kinase domain of *MPS1* do not show a cell cycle arrest with a monopolar spindle, but instead carry out an aberrant and lethal mitosis at restrictive temperature (Winey *et al.*, 1991). This failure to arrest mimics strains doubly mutant in *CDC31* and a checkpoint gene, such as *MAD2* (Weiss and Winey, 1996). Initially, it seemed possible that the unique aberrant SPB in *mps1⁻* mutants failed to trigger the checkpoint pathway, but analysis of cells synchronized after SPB duplication revealed that Mps1p is required in the spindle assembly checkpoint (Weiss and Winey, 1996). Recently, yeast Mps1p has been localized to kinetochores, consistent with its function in the spindle assembly checkpoint (Castillo *et al.*, 2002).

A second clue to the involvement of Mps1p in the spindle assembly checkpoint came from the overexpression phenotype in yeast. High levels of Mps1p lead to a cell cycle arrest in metaphase in the apparent absence of spindle damage (Hardwick *et al.*, 1996). Deletion of any of the checkpoint genes (*MAD1*, 2, 3 or *BUB1*, 3) blocks the ability of overexpressed Mps1p to arrest the cell cycle indicating that the arrest required the spindle assembly checkpoint pathway and that the known members of the pathway are downstream of Mps1p (Hardwick *et al.*, 1996). The spindle assembly checkpoint seems to be triggered in response to at least two different spindle defects; lack

of kinetochore attachment and lack of kinetochore tension. Recently, the yeast Aurora kinase, *IPL1*, has been implicated in the spindle assembly checkpoint in yeast but only in response to a lack of kinetochore tension (Biggins and Murray, 2001). It was shown that *ipl1⁻* mutant strains do not arrest in the presence of over-expressed Mps1p, suggesting a role for *IPL1* in the *MPS1* overexpression arrest. A final interesting note is that *MPS1* overexpression arrest, unlike other arrest states mediated by the spindle assembly checkpoint, does not require an intact kinetochore. Over-expression of Mps1p in a *ndc10-1* strain which lacks kinetochores still causes an arrest, suggesting that the spindle assembly checkpoint pathway can be activated in the absence of kinetochores (Fraschini *et al.*, 2001).

A molecular marker for activation of the spindle assembly checkpoint in budding yeast is hyperphosphorylation of Mad1p (Hardwick and Murray, 1995). Mps1p overexpression leads to very high levels of Mad1p phosphorylation whereas inhibition of Mps1p with a mutant allele leads to reduced Mad1p phosphorylation, indicating that Mps1p controls Mad1p phosphorylation state *in vivo* (Hardwick *et al.*, 1996). This may represent a direct interaction because Mps1p is able to phosphorylate Mad1p *in vitro*. In summary, Mps1p is required early in the spindle assembly checkpoint and can activate the pathway when overexpressed, suggesting that it may be the limiting step in checkpoint activation. How Mps1p is activated in mitotic cells to trigger the checkpoint as well as the identity of Mps1p substrate(s) for its function in the checkpoint remain to be determined.

A screen for fission yeast genes that arrest cells in metaphase when overexpressed led to the identification of the *S. pombe* *MPS1* ortholog, called *mph1⁺* (He *et al.*, 1997). This gene is not essential in fission yeast as discussed above, but the deletion strains show defects in the spindle assembly checkpoint. Furthermore, a partial rescue of budding yeast *MPS1* phenotypes by *mph1⁺* has been observed as well as full rescue of the checkpoint defect in *mph1⁺* by *MPS1* (He *et al.*, 1997). *mph1p* (as well as *bub1p*) is required for *mad3p* recruitment to the kinetochore; an early, critical step in the checkpoint pathway in fission yeast (Millband and Hardwick, 2002).

Similar to the observations in yeasts, Mps1p kinase has been implicated in the spindle assembly checkpoint in vertebrate cells and in *Xenopus* egg extracts (Abrieu *et al.*, 2001; TG Yen, personal communication; Stucke *et al.*, 2002). The kinase localizes to kinetochores in both human and mouse cells (TG Yen, personal communication; Fisk and Winey, 2001; Stucke *et al.*, 2002). Functional analyses in human cells show that when the kinase is inhibited, cells do not arrest properly in the presence of nocodazole (a microtubule destabilizing drug) indicating a defect in the spindle checkpoint (Abrieu *et al.*, 2001; TG Yen, personal communication; Stucke *et al.*, 2002). Both hMps1p antibody microinjection and small interfering RNA (siRNA) experiments in HeLa cells show that hMps1p

is required to trigger and/or maintain a checkpoint arrest in response to microtubule depolymerization (TG Yen, personal communication; Stucke *et al.*, 2002). Similarly, depletion of xMps1p (*Xenopus*) leads to failure of the spindle checkpoint in extracts (Abrieu *et al.*, 2001). The *Xenopus* enzyme associates with kinetochores and the kinase-dead protein also blocks the function of the spindle checkpoint in this system. The kinetochore recruitment of several checkpoint components was monitored in *Xenopus* extracts that had been depleted for xMps1p (Abrieu *et al.*, 2001). Interestingly, and consistent with the yeast data, is the finding that Mps1p must act early in the pathway because Mad1p, Mad2p, and CENP-E are not found at kinetochores in extracts depleted for xMps1p (Abrieu *et al.*, 2001). Localization of hMps1p to kinetochores may depend on CENP-E in HeLa cells, however, localization of CENP-E does not seem to require hMps1p (TG Yen, personal communication).

The MPS1 family of kinases clearly function in the spindle assembly checkpoint in all organisms analysed (Figure 3). Mutations in the genes in this pathway have been reported in a few cancers, but no mutations in *MPS1* have been found (Cahill *et al.*, 1999). Although the data on Mps1p is scant in some systems, the consistent findings are that the protein is at kinetochores and that kinase activity is necessary for its function in the checkpoint. Furthermore, the Mps1p kinase acts early in the checkpoint pathway where tested. Because of the different systems used, it is difficult to determine exactly where Mps1p acts in the pathway. In *Xenopus* and possibly yeasts, MPS1 kinases appear to be required very early in checkpoint activation. If indeed Mps1p acts in the first steps in the checkpoint pathway, what Mps1p responds to and how it is activated become central questions in the function of this pathway.

Other functions of MPS1 kinases

Like other protein kinases that control mitotic progression, MPS1 kinases are likely to have functions beyond their roles in pole duplication and the spindle assembly checkpoint. One potential role was revealed by a two-hybrid analysis with the budding yeast gene *MPS1* that identified the *MOB1* gene (Luca, 1998). Mob1p, a member of the Mitotic Exit Network (MEN, reviewed in Hoyt, 2000) acts to activate the Dbf2p protein kinase for completion of mitosis (Mah *et al.*, 2001). As part of its function, Mob1p is transiently localized to the SPB and to the budneck, the site of cytokinesis in this yeast (Luca *et al.*, 2001). Mob1p can be co-immunoprecipitated with Mps1p from yeast extracts and is phosphorylated by Mps1p *in vitro*, but the function of the interaction is unknown (Luca, 1998). Strains doubly mutant for specific alleles of *MOB1* and *MPS1* show an increase-in-ploidy phenotype (a phenotype common in mutant strains defective in SPB duplication; reviewed in Chial and Winey, 1999; Luca, 1998). It has been proposed that this interaction may be the basis of a 'licensing' event that will allow SPB duplication in the subsequent cell cycle, similar to the licensing of chromosomal DNA replication. Alternatively, Mps1p may have a more direct role in the exit pathway via the inhibition of the Dbf2p kinase (Fesquet *et al.*, 1999). However, these results are based on the use of GAL-Mps1p, which is expressed at high levels in mitotically arrested cells but which may not behave the same in other parts of the cell cycle. It is unlikely that this role is essential, as Weiss and Winey have shown that cells released from HU induced S phase arrest complete the cell cycle without Mps1p activity (Weiss and Winey, 1996).

The analysis of Mps1p function in meiosis revealed a requirement for the kinase in chromosome segregation beyond its checkpoint role (Straight *et al.*, 2000). Genetic interactions with mutations in kinetochore and spindle components have hinted that Mps1p may have a role in chromosome segregation in mitotic cells (Jones *et al.*, 1999). While strains containing other checkpoint mutations (*mad1Δ* or *mad2Δ*) have a modest defect in Meiosis I (Shonn *et al.*, 2000), strains mutant in *MPS1* exhibit chromosome missegregation events in Meiosis I and II that are so severe that some chromosomal DNA never gets packaged into spores (Straight *et al.*, 2000). A possibility is that the early role of Mps1p in the spindle assembly checkpoint may include proper assembly of a functional kinetochore (Jones *et al.*, 2001), such that it has the ability to signal an arrest when there is a defect in chromosome attachment.

Examination of the yeast *MPS1* mutants in meiotic cells showed that *MPS1* is required for both rounds – Meiosis I and II – of SPB duplication (Straight *et al.*, 2000). An unexpected finding was that Mps1p is also required for spore wall formation (Straight *et al.*, 2000). The formation of spores in budding yeast is a post meiotic event in which the individual meiotic products are packaged into spores. A transcriptional program normally activated during meiosis and spore

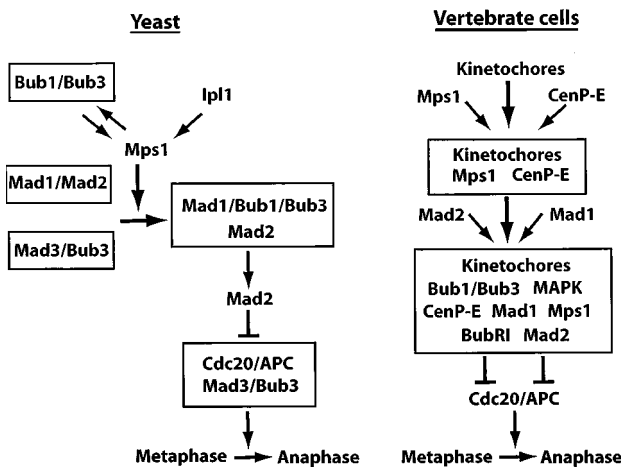


Figure 3 The spindle assembly checkpoint in yeast and vertebrate cells. Schematic diagram of the spindle assembly checkpoint in both yeast (similar to Brady and Hardwick, 2000) and vertebrate cells. Boxes designate protein complexes. Only recruitment of vertebrate checkpoint proteins shown to depend on Mps1p are indicated (see text for details). Other checkpoint proteins are listed in the final kinetochore complex

formation is under the control of several protein kinases, including a MAP/ERK kinase homolog, Smk1p (Krisak *et al.*, 1994). *mps1*⁻ mutant strains show defects in this transcriptional program similar to *smk1*⁻ mutant strains, but the exact role of Mps1p in this regulatory network is unknown (Straight *et al.*, 2000). This is the only known function of the kinase that does not involve chromosome segregation and the microtubule cytoskeleton.

Control of Mps1p activity

The important and diverse roles of members of the MPS1 kinase family suggest that there must be significant regulation of the kinase. Indeed, overexpression of the kinase leads to defects in centrosome duplication in mammalian cells and the spindle assembly checkpoint in yeast cells, as does loss of function of the kinase (Fisk and Winey, 2001; Hardwick *et al.*, 1996). As one might expect, the kinase appears to be regulated at a number of levels.

In a myriad of microarray experiments in budding yeast, *MPS1* shows little variation in levels of transcription (available at <http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=mps1> (Ball *et al.*, 2000)). *MPS1* mRNA levels are low and unchanged during the mitotic cell cycle, but do show an induction late in meiosis that may be necessary for the protein's role in spore formation (Chu *et al.*, 1998). mRNA levels for *MPS1* also decrease in cells arrested in G1 by mating factor by an unknown mechanism (Poch *et al.*, 1994), and this presumably contributes to blocking SPB duplication at the specific SPB intermediate needed for mating. In vertebrates, the mRNAs for *MPS1* orthologs are found in all proliferating tissue, normal or transformed, with the exception of a very few cancer cell lines (Mills *et al.*, 1992). This finding is consistent with the role(s) of the MPS1 family members in mitotic progression. Studies using T98G cells released from arrests at various points in the cell cycle showed that *hMPS1* mRNA levels are low at G1/S and steadily increase throughout S phase. *hMPS1* mRNA levels peaked at late G2/M, and dropped upon cells entering G1 (Hogg *et al.*, 1994). Similarly, these authors showed that hMps1p protein levels and kinase activity peaked in mitosis, a result recently confirmed in HeLa cells (Stucke *et al.*, 2002).

As cells enter mitosis, the kinase activity of hMps1p increases to a greater extent than the protein level clearly indicating post-transcriptional control of the kinase (Stucke *et al.*, 2002). Mps1p kinases are phosphoproteins in all cell types examined, and become hyperphosphorylated in checkpoint arrested cells (TG Yen, personal communication; Schutz and Winey, 1998). Upon release from a mitotic arrest, hMps1p becomes dephosphorylated when cells enter anaphase (TG Yen, personal communication). This hyperphosphorylation is likely to contribute to the high levels of activity seen in checkpoint arrested human cells and yeast cells. We have mutated some phosphorylated

residues in yeast Mps1p that render the enzyme inactive, but a complete description of the phosphorylation sites has not been done and the function(s) of phosphorylation is not known.

In addition to phosphorylation, many kinases are activated by an associated regulatory subunit. There is no evidence for such a regulatory subunit for Mps1p kinases, but genetic evidence in yeast indicates that Mps1p does require molecular chaperones for its function. In particular, Mps1p has reduced activity in *cdc37* strains (Schutz *et al.*, 1997). *CDC37* encodes the yeast version of the p50 protein kinase targeting subunit of the Hsp90 chaperone complex (Stepanova *et al.*, 1996). *CDC37* has been shown to interact with a number of protein kinases in yeast and is thought to have some general role in kinase activation. A requirement for chaperones in the activation of the vertebrate MPS1 kinases has not been tested.

Finally, protein degradation is an important component of cell cycle regulation and the turnover of MPS1 kinases may be an important mechanism for their control. In NIH3T3 cells, mMps1p protein levels in S phase are known to be dependent on CDK2 activity (Fisk and Winey, 2001). The loss of mMps1p in S phase arrested cells upon CDK2 inhibition may explain why mMps1p driven centrosome reduplication requires CDK2 activity. Also, the loss of mMps1p was shown to be proteasome dependent (Fisk and Winey, 2001). mMps1p is found at centrosomes in cells treated with both CDK2 and proteasome inhibitors suggesting that the major contribution of CDK is to stabilize mMps1p. This stabilization may be achieved by direct phosphorylation of mMps1p by CDK (Fisk and Winey, 2001). A survey of a number of cell lines shows similar control of MPS1 kinase turnover (H Fisk, C Mattison and M Winey, unpublished observations). Yeast Mps1p has a short half-life and it is possible that turnover contributes to its control as well (Schutz *et al.*, 1997). In fact, the half-life is short enough that it is unlikely that the same pool of enzyme executes SPB duplication and the checkpoint functions of the kinase. Thus, the possibility exists that the two different pools of Mps1p could be processed and modified differently for each of its roles.

One type of regulation that is not apparent is an activating phosphorylation event, although autophosphorylation may contribute to activation. Recombinant Mps1p produced in bacteria is active in kinase assays, showing significant autophosphorylation and substrate phosphorylation (Douville *et al.*, 1992; Lindberg *et al.*, 1993; Mills *et al.*, 1992). Rigorous testing for subtle changes in Mps1p activity has not been done, and understanding of Mps1p's substrate specificity is not yet possible. Nonetheless, if Mps1p is constitutively active at some level, then temporal and spatial control will be important in its regulation.

Prospectus and outstanding questions

The MPS1 family is emerging as an important protein kinase family involved in regulating several events

during mitotic progression. Identifying its precise functions at a mechanistic level is an immediate goal in the field. It will be interesting also to determine which roles are widely conserved, and which are not. To help address these questions significant work needs to be done in determining how Mps1p expression and activity is controlled. Finally, as with any protein kinase, the identification of substrates is critical, particularly those substrates that reveal its specific functions in the various stages of mitosis. While there is much to do, there is no doubt that MPS1 kinases are a widely conserved family of kinases intimately involved in the control of mitosis.

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