# Killing two birds with one stone: how budding yeast Mps1 controls chromosome segregation and spindle assembly checkpoint through phosphorylation of a single kinetochore protein

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**Abstract**

During mitosis, the identical sister chromatids of each chromosome must attach through their kinetochores to microtubules emanating from opposite spindle poles. This process, referred to as chromosome biorientation, is essential for equal partitioning of the genetic information to the two daughter cells. Defects in chromosome biorientation can give rise to aneuploidy, a hallmark of cancer and genetic diseases. A conserved surveillance mechanism called spindle assembly checkpoint (SAC) prevents the onset of anaphase until biorientation is attained. Key to chromosome biorientation is an error correction mechanism that, by disengaging faulty kinetochore-microtubule connections, allows kinetochores to establish proper bipolar attachments. Error correction relies on the Aurora B and Mps1 kinases that also promote SAC signalling, raising the possibility that they are part of a single sensory device responding to improper attachments and concomitantly controlling both their disengagement and a temporary mitotic arrest.

In budding yeast Aurora B and Mps1 promote error correction independently from one another, but while the substrates of Aurora B in this process are at least partially known, the mechanism underlying the involvement of Mps1 in the error correction pathway is unknown. Through the characterization of a novel *mps1* mutant and an unbiased genetic screen for extragenic suppressors, we recently gained evidence that a common mechanism based on Mps1-dependent phosphorylation of the Knl1/Spc105 kinetochore scaffold and subsequent recruitment of the Bub1 kinase is critical for the function of Mps1 in chromosome biorientation as well as for SAC activation (Benzi et al., EMBO Rep. 2020 Apr 19:e50257. doi: 10.15252/embr.202050257).

## Introduction

Chromosome segregation is a vulnerable, error-prone process that must be tightly regulated in time and space. Several requirements must be fulfilled in order for daughter cells to get an equal chromosome complement. First, chromosomes must be faithfully duplicated into identical sister chromatids through DNA replication. Second, sister chromatids must be glued together by sister chromatid cohesion, which allows cells to distinguish genetically identical from distinct chromosomes. Third, sister chromatids need to attach via their kinetochores to microtubules emanating from opposite spindle poles, a process referred to as chromosome biorientation (reviewed in Bloom and Yeh, 2010). If this fails, a surveillance mechanism called Spindle Assembly Checkpoint (SAC) temporarily halts cell cycle progression in metaphase to allow error correction. Conversely, when all chromosomes are bipolarly attached, SAC is satisfied and anaphase can shortly ensue.

SAC detects the lack of attachment between spindle microtubules and kinetochores (reviewed in Maresca and Salmon, 2010), which are large protein assemblies residing at the centromere of each chromosome (reviewed in Musacchio and Desai 2017). Unattached kinetochores generate an alert signal that ultimately leads to the formation of a Mitotic Checkpoint Complex (MCC), made by Bub3, Mad2, BubR1 (Mad3 in yeast) and Cdc20, that binds to and inhibits the E3 ubiquitin ligase Anaphase Promoting Complex bound to its activator Cdc20 ($APC^{Cdc20}$). In turn, stabilisation of $APC^{Cdc20}$ targets, such as securin and cyclin B, prevents sister chromatid separation and mitotic exit, thereby imposing a reversible metaphase arrest that provides the time for correction of improper kinetochore-microtubule connections (reviewed in Musacchio, 2015 ; Sacristan and Kops, 2015).

The dual-specificity mitotic kinase Mps1 (Monopolar spindle 1) is a key player in SAC signaling. Mps1 was first identified in budding yeast (Winey et al. 1991) due to its involvement in the duplication of spindle pole bodies (SPBs), the functional equivalents of centrosomes in animal cells.

While the involvement of Mps1 in centrosome duplication in eukaryotic cells other than yeast is controversial (Stucke et al. 2002; Fisk et al. 2003; Kwiatkowski et al. 2010), Mps1 is almost universally implicated in SAC activation (reviewed in Pachis and Kops, 2018). Mps1 works at the apex of SAC signaling through phosphorylation of the kinetochore protein Knl1/Spc105 on its N terminal MELT repeats, which in turn is essential for the kinetochore recruitment of the Bub1-Bub3 complex (London et al. 2012; Shepperd et al. 2012; Yamagishi et al. 2012; Primorac et al. 2013). Mps1 also phosphorylates Bub1 and Mad1, allowing their interaction and kinetochore recruitment of Mad2 that acts as a catalyzer for MCC assembly (Faesen et al., 2017; Ji et al., 2018; London and Biggins, 2014; Mora-Santos et al., 2016; Moyle et al., 2014).

**Coupling chromosome biorientation with the Spindle Assembly Checkpoint**

During the SAC induced-metaphase arrest, cells rectify the tensionless kinetochore-microtubule attachments with the aim of establishing chromosome biorientation. An error correction mechanism involves the continuous detachment of improper attachments to provide kinetochores with further opportunities to capture microtubules (reviewed in Lampson and Grishchuk, 2017). When tension across kinetochores is finally established, bipolar attachments are stabilized and chromosomes congress to the cell's equator, due to the equilibrium between pulling and cohesive forces (by microtubules and sister chromatid cohesion, respectively).

A key player in the error correction pathway is the Chromosomal Passenger Complex (CPC). The CPC is composed of the Aurora B kinase, the inner centromere protein INCENP, Survivin and Borealin (Ipl1, Sli15, Bir1 and Nbl1, respectively, in budding yeast) (reviewed in Carmena et al., 2012). Aurora B detaches improper kinetochore-microtubule connections by progressively phosphorylating Ndc80/Hec1, the subunit of the NDC80 complex that directly interacts with microtubules (Cheeseman et al. 2006; DeLuca et al. 2006; Ciferri et al. 2008; Alushin et al. 2010; Tooley et al. 2011; Zaytsev et al. 2014, 2015). Moreover, Aurora B/Ipl1 phosphorylates the DAM1 complex in budding yeast and its functional homolog SKA complex in mammalian cells (Chan et al., 2012; Cheeseman et al., 2002; Kalantzaki et al., 2015; Lampert et al., 2010; Tien et al., 2010), which couples chromosome mouvement to microtubule depolymerization (Abad et al., 2014; Asbury et al., 2006; Grishchuk et al., 2008; Lampert et al., 2010; Schmidt et al., 2012; Tien et al., 2010; Welburn et al., 2009; Westermann et al., 2006).

Besides correcting improper kinetochore-microtubule connections, Aurora B is also part of SAC signaling (reviewed in Krenn and Musacchio, 2015). On one side, it generates unattached kinetochores that are sensed by SAC (Pinsky et al. 2006). On the other, it facilitates the rapid recruitment of Mps1 to kinetochores (Santaguida et al., 2011; Saurin et al., 2011) and phosphorylates the RVSF motif in KNL1 (Liu et al. 2010). The latter hampers the binding of the PP1 phosphatase, which is required for SAC silencing (Liu et al., 2010; Pinsky et al., 2006; Rosenberg et al., 2011; Vanoosthuyse and Hardwick, 2009). Thus, Aurora B contributes to chromosome biorientation by both promoting error correction and sustaining SAC signaling. Similarly, The Mps1 kinase has been involved in the correction of faulty kinetochore-microtubule attachments, in addition to triggering SAC activity (Jones et al., 2005;

Maure et al., 2007; Santaguida et al., 2010; Hewitt et al., 2010; Maciejowski et al., 2010). However, its role in the error correction pathway is far from being understood. While in mammalian cells Mps1 seems to work in concert with Aurora B (Vigneron et al. 2004; Jelluma et al. 2008; Saurin et al. 2011; Santaguida et al. 2011), in yeast the two kinases appear to play independent roles (Maure et al. 2007; Storchová et al. 2011). In addition, while in mammalian cells the SKA complex has been proposed to be a relevant Mps1 substrate in the error correction pathway (Maciejowski et al., 2017), in the budding yeast *S. cerevisiae* the critical targets of Mps1 in this process remain to be identified. Indeed, although *S.c.*Mps1 phosphorylates Dam1 and Ndc80 on several residues, none of these phosphorylations is required for chromosome biorientation (Shimogawa et al. 2006; Kemmler et al. 2009; Kalantzaki et al. 2015). The identification of the critical targets of *S.c.*Mps1 in chromosome biorientation is not an easy task, and is complicated by the essential function of *S.c.*Mps1 in SPB duplication and spindle assembly (Weiss and Winey 1996). Indeed, the initial discovery that Mps1 is involved in the error correction pathway was based on a complex experimental set-up (Maure et al. 2007).

**A novel genetic tool to dissect the function of Mps1 in chromosome biorientation**

We have recently characterized a novel temperature-sensitive mutant, named *mps1-3,* that bears a single substitution of serine 635 to phenylalanine. The *mps1-3* mutant is lethal at temperatures above 32°C, and at temperatures ranging from 32°C to 34°C is severely defective in chromosome biorientation but proficient in SPB duplication and spindle elongation (Benzi et al. 2020), unlike the majority of *mps1* mutants (Weiss and Winey 1996; Schutz and Winey 1998; Castillo et al. 2002; Araki et al.

2010). Contextually, *mps1-3* cells are also SAC-deficient, which allows them to progress through the cell cycle in the face of chromosome misalignment, thus accumulating massive aneuploidy. We could attribute lack of chromosome biorientation in *mps1-3* cells to faulty correction of improper attachments, rather than to a failure to establish kinetochore-microtubule connections, in agreement with earlier conclusions on Mps1 inhibition through an analogue-sensitive mutant (Jones et al. 2005; Maure et al. 2007). Consistent with defects in the error correction pathway, in *mps1-3* cells sister chromatids co-segregate preferentially toward the bud (Benzi et al. 2020), where the old SPB usually migrates during anaphase (Pereira et al. 2001). This phenotype had been previously described for *ipl1* (Aurora B) mutants and is likely due to a delay in the gain of microtubule-nucleating activity of the new versus the old SPB, thereby biasing kinetochore attachment to the old, mature SPB (Tanaka et al. 2002).

To our surprise, the *mps1-3* mutation, which hits the kinase domain of the protein (aa 440-720), increases the *in vitro* kinase activity of Mps1 relative to the wild type protein. However, the Mps1-3 does not localize at kinetochores at restrictive temperatures. Although Mps1 can sustain SAC signaling away from kinetochores under non-physiological conditions (Maciejowski et al., 2017; Fraschini et al., 2001; Yuan et al., 2017), its kinetochore recruitment clearly contributes to proficient chromosome biorientation and SAC signaling in normal cells (Jelluma et al. 2010; Saurin et al. 2011; Heinrich et al. 2012; Nijenhuis et al. 2013; Zhu et al. 2013; Aravamudhan et al. 2016). Thus, the SAC and chromosome segregation defects of *mps1-3* mutant cells likely stem from the lack of phosphorylation of critical Mps1 substrates at kinetochores. Consistently, phosphorylation of known Mps1 substrates at kinetochores, such as Spc105/Knl1, Bub1, and Mad1 (Shepperd et al. 2012;

Yamagishi et al. 2012; Primorac et al. 2013; London and Biggins 2014; Moyle et al. 2014; Mora-Santos et al. 2016; Faesen et al. 2017; Qian et al. 2017; Ji et al. 2018) is markedly compromised in *mps1-3* mutant cells. Additionally, artificial anchoring of Mps1 to the kinetochore protein Mtw1 partially restores proper chromosome segregation in *mps1-3* cells (Benzi et al. 2020).

The domain responsible for Mps1 kinetochore localization lies at the N-terminus of the protein, far from the catalytic domain (Ji et al.; Liu et al. 2003; Stucke et al. 2004; Kemmler et al. 2009; Araki et al. 2010; Hached et al. 2011; Nijenhuis et al. 2013; Hiruma et al. 2015; Maciejowski et al. 2017). Thus, it is unlikely that the *mps1-3* mutation interferes directly with Mps1 binding to its kinetochore receptor(s). Nonetheless, Mps1 has been proposed to accelerate its own kinetochore turnover through autophosphorylation (Sliedrecht et al. 2010; Kwiatkowski et al. 2010; Hewitt et al. 2010; Santaguida et al. 2010; Jelluma et al. 2010; Wang et al. 2014; Dou et al. 2015; Koch et al. 2019; Hayward et al. 2019). Furthermore, activation of the Mps1 kinase leads to extensive conformational changes in the protein (Combes et al. 2018), which in turn could mask its kinetochore-binding regions. Thus, the increased kinase activity of Mps1-3 could reduce its retention time at kinetochores, thereby impairing SAC signaling and chromosome biorientation. We attempted to test directly this hypothesis by introducing into the *mps1-3* allele the analog-sensitive *mps1-as1* mutation, which inhibits the kinase in the presence of the ATP analog inhibitor 1-NM-PP1 (Jones et al. 2005). However, the double mutant turned out to be lethal even in the absence of the analog (our unpublished data), making this approach inconclusive. The characterization of the internal suppressors that we identified (see below) might provide us with alternative tools to test the contribution of the hyperactivity of the Mps1-3 kinase in its own kinetochore turnover.

**The Spc105/Knl1 kinetochore scaffold as a critical target of Mps1 in chromosome biorientation beyond SAC signaling**

Out of the two processes that are affected by the *mps1-3* mutation, i.e. SAC signaling and chromosome biorientation, only the latter is essential for cell viability and accounts for the lethality of *mps1-3* cells at high temperatures. This makes the *mps1-3* mutant a valuable genetic tool to underpin the molecular bases underlying the involvement of Mps1 in the error correction pathway. We therefore exploited the potential of budding yeast as a powerful genetic tool to identify spontaneous suppressors that enable *mps1-3* cells to divide at 34°C. This unbiased genetic screen was instrumental to our current model envisioning a common Mps1 substrate (Spc105/Knl1, i.e. the stone) in the control of both chromosome biorientation and SAC (i.e. the two birds).

Through our genetic screen we isolated several intragenic suppressors that carried second site mutations within the *mps1-3* allele. These were all missense mutations in the catalytic kinase domain. Their future characterization might reveal important insights into the control of Mps1 activity and kinetochore recruitment. We also isolated extragenic suppressors that bore, instead, a suppressing mutation in genes other than *MPS1*. Strikingly, all of them rescued both chromosome segregation and SAC defects of *mps1-3* cells, suggesting that a common mechanism underlies the role of Mps1 in these processes. Among these suppressors, we found mutations in *SPC105*, which codes for the kinetochore protein Spc105/Knl1, and *GLC7*, which codes for the catalytic subunit of the protein phosphatase PP1. Importantly, the *spc105* suppressing mutations hit the motif (RVSF) for Spc105 binding to PP1, while the *GLC7* suppressors carried a missense mutation of Phe256, which resides in the

hydrophobic groove involved in PP1 interaction with partners carrying the RV/IXF consensus motif (Wu and Tatchell 2001). As mentioned above, Mps1-dependent phosphorylation of Spc105/Knl1 at its MELT repeats recruits the Bub3-Bub1 complex and is critical for SAC signaling (London et al. 2012; Shepperd et al. 2012; Yamagishi et al. 2012; Primorac et al. 2013). Upon chromosome biorientation, Spc105/Knl1 phosphorylation is reversed by the phosphatase PP1, which binds the RVSF motif and silences the SAC (Pinsky et al. 2009; Vanoosthuyse and Hardwick 2009; Liu et al. 2010; Rosenberg et al. 2011; Meadows et al. 2011; London et al. 2012; Moura et al. 2017). Importantly, the *spc105* and *GLC7* suppressors that we isolated did not restore Mps1 kinetochore localization, strongly suggesting that PP1 opposes Mps1 activity at kinetochores for both proper chromosome biorientation and SAC signaling. The antagonism between Mps1 and PP1 has been well characterized for SAC, while its involvement in the error correction pathway had not been established. Altogether, our data have two important implications: first, the same Mps1-dependent sensory device may be used for both correction of improper kinetochore-microtubule attachments and SAC signaling, in line with previous proposals (Musacchio 2011; Caldas et al. 2013); second, PP1 is expected to stabilize kinetochore-microtubule attachments in yeast, as it does in human cells (Liu et al. 2010).

On the basis of our results, we wondered if Spc105 phosphorylation and subsequent Bub1 recruitment could be the main function of Mps1 in the error correction pathway, as it is in SAC activation. Consistent with this idea, Bub1 is no longer recruited to kinetochores in *mps1-3* mutant cells, while it regains kinetochore binding in the suppressors. Furthermore, artificial tethering of Bub1 to Spc105 is sufficient to restore balanced chromosome segregation and partial SAC signaling in *mps1-3* cells (Benzi et al. 2020). Thus, our data strongly imply that Mps1 promotes the error

correction pathway and SAC activity via a common mechanism that relies on Spc105/Knl1 phosphorylation (Fig. 1). Along this line, phosphorylation of other Mps1 kinetochore targets, such as Dam1 and Ndc80, turned out to be dispensable for equal chromosome segregation (Shimogawa et al. 2006; Kemmler et al. 2009; Kalantzaki et al. 2015).

The main conclusion of our paper is consistent with several published observations: a *spc105-6A* yeast mutant with unphosphorylatable MELT repeats is defective in Bub1 kinetochore recruitment and displays chromosome missegregation (London et al. 2012); phospho-mimicking mutations in the MELT repeats of Spc7 (the fission yeast homologue of Spc105/Knl1) partially suppress the chromosome biorientation defects of fission yeast *mps1Δ* cells, though in fission yeast Mps1 only modestly contributes to chromosome biorientation (Yamagishi et al. 2012). Finally, Knl1 depletion in human cells impairs Aurora B activity and chromosome alignment on the metaphase plate (Caldas et al. 2013).

**Is Bub1 kinetochore recruitment the only function of Mps1 in chromosome biorientation?**

Bub1 has been extensively involved in chromosome biorientation through phosphorylation of T120 of histone H2A (S121 in yeast), which in turn recruits shugoshin proteins (Sgo1 in budding yeast) to pericentromeres (Fernius and Hardwick 2005; Kawashima et al. 2010; Yamagishi et al. 2010; Liu et al. 2015). Shugoshins, in turn, promote recruitment of the CPC and biorientation of sister chromatids (Indjeian et al. 2005; Kawashima et al. 2007; Huang et al. 2007; Kiburz et al. 2008; Tsukahara et al. 2010; Yamagishi et al. 2010; Verzijlbergen et al. 2014; Liu et al. 2015). Consistent with Bub1 being required for Sgo1 accumulation at

pericentromeres, localization of Sgo1 at kinetochores is compromised in *mps1-3* mutant cells. However, the synthetic lethality between the *mps1-3* mutation and *SGO1* deletion suggests that Mps1 could have at least one additional function in chromosome biorientation, besides recruiting Bub1 to kinetochores. Indeed, Bub1 and Sgo1 are not essential proteins, in contrast to Mps1. Furthermore, artificial tethering of Bub1 to Spc105 cannot fully rescue the chromosome biorientation defects and temperature-sensitivity of *mps1-3* cells (Benzi et al. 2020), suggesting that other Mps1 substrates and/or other factors binding to Spc105 might intervene. One intriguing hypothesis, which would be supported by our genetic data, is that Mps1 could keep PP1 inhibited at kinetochores. PP1, in turn, stabilizes bipolar attachments (Liu et al. 2010).

Another interesting candidate that might play a function complementary to Bub1 in chromosome biorientation, downstream of Mps1, is the microtubule rescue factor Stu1, which is a member of the CLASP family (Al-Bassam and Chang 2011). At kinetochores, Stu1 stabilizes microtubules, and, consistently, it gets sequestered at unattached kinetochores to facilitate their own capturing (Funk et al. 2014). Stu1 sequestration at unattached kinetochores requires Mps1 and Spc105 phosphorylation of its MELT repeats, but not Bub1 (Kolenda et al. 2018), suggesting that Stu1 and Bub1 might cooperate at Spc105 to achieve successful bipolar attachment. Stu1 recruits Stu2, a microtubule-associated protein of the XMAP215 family, to polymerize kinetochore-microtubules (Vasileva et al. 2017). Remarkably, Stu2 has been recently proposed to mediate an error correction pathway independent of Aurora B/Ipl1 in budding yeast (Miller et al. 2016, 2019). Whether and how Mps1 modulates Stu1 and/or Stu2 function at kinetochores to achieve

chromosome biorientation is an important question that deserves future investigations.

Another important question that remains open is whether an interplay between *S.c.*Mps1 and the CPC in the error correction pathway can be completely ruled out. As already mentioned, in budding yeast Mps1 and Ipl1 were proposed to operate independently from one another (Maure et al. 2007; Storchová et al. 2011). Our data are consistent with this view. First, phosphorylation of the known kinetochore substrate of Ipl1/Aurora B Dam1 is not affected in *mps1-3* cells (Benzi et al. 2020). Similarly, Mps1 inhibition does not perturb phosphorylation of Aurora B substrates in human cells (Hewitt et al. 2010; Maciejowski et al. 2010; Santaguida et al. 2010). Second, the temperature-sensitivity of *mps1-3* cells cannot be suppressed by either the *NDC80-13D* phospho-mimicking mutant allele where all Mps1-dependent phosphorylation sites of Ndc80 have been replaced by aspartate (Kemmler et al. 2009), or the *DAM1-S221F* allele (also known as *DAM1-765,* (Shimogawa et al. 2006), which was previously found to suppress the temperature-sensitivity of *ipl1-321* mutant cells (Shimogawa et al. 2010; our unpublished data). Third, mutations in the CPC subunit Sli15 that activate Ipl1/Aurora B and rescue the sickness of *bir1Δ*, *bub1Δ* and *sgo1Δ* cells (Campbell and Desai 2013) do not suppress the lethality and chromosome segregation defects of *mps1-3* cells at high temperature (Benzi et al. 2020). Thus, we favour the hypothesis that Mps1 sets in motion an error correction mechanism that does not involve Aurora B/Ipl1. However, it remains possible that, while differing in their respective upstream targets, the Mps1- and Ipl1-dependent error correction pathways ultimately converge on shared downstream effectors to destabilise flawed attachments. Future work will certainly shed light on this exciting possibility.

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Figure 1

Benzi and Piatti, Fig. 1