The Aurora B Kinase Promotes Inner and Outer Kinetochore Interactions in Budding Yeast

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ABSTRACT The kinetochore is the macromolecular protein complex that mediates chromosome segregation. The Dsn1 component is crucial for kinetochore assembly and is phosphorylated by the Aurora B kinase. We found that Aurora B phosphorylation of Dsn1 promotes the interaction between outer and inner kinetochore proteins in budding yeast.

ACCURATE chromosome segregation is essential to avoid aneuploidy and maintain genomic stability (Holland and Cleveland 2012). Segregation is directed by the kinetochore, the macromolecular complex that interacts with spindle microtubules during cell division (Westermann *et al.* 2007; Cheeseman and Desai 2008; Bloom and Joglekar 2010). The kinetochore is composed of multiple copies of distinct subcomplexes that assemble into a macromolecular structure estimated to be >5 MDa (Joglekar *et al.* 2006; Lawrimore *et al.* 2011; Gonen *et al.* 2012). The inner kinetochore contains proteins that closely associate with centromeric DNA, whereas the outer kinetochore contains the proteins that bind to microtubules. At the base of the kinetochore, the CENP-A centromeric histone H3 variant forms a specialized chromatin environment essential for recruiting other kinetochore proteins such as the CCAN (**c**onstitutive **c**entromere-**a**ssociated **n**etwork) (Buscaino *et al.* 2010; Perpelescu and Fukagawa 2011). These inner kinetochore components are essential for the assembly of the outer kinetochore, which includes the KMN (**K**NL1, **M**is12, **N**dc80 complexes) network that exhibits robust microtubule-binding activity (Cheeseman *et al.* 2006; Okada *et al.* 2006; Powers *et al.* 2009; Tanaka *et al.* 2009; Akiyoshi *et al.* 2010; Carroll *et al.* 2010; Przewloka *et al.* 2011; Screpanti *et al.* 2011). Although the core kinetochore components have been identified, the requirements for their assembly onto the centromere to form the macromolecular kinetochore structure are still unknown (Gascoigne and Cheeseman 2011).

Dsn1, a subunit of the Mis12 complex, is essential for kinetochore assembly (Kline *et al.* 2006), and may be a keystone for outer kinetochore assembly (Cheeseman and Desai 2008; Davies and Kaplan 2010; Maskell *et al.* 2010; Petrovic *et al.* 2010; Malvezzi *et al.* 2013). The Ipl1/Aurora B kinase is known to destabilize erroneous kinetochore-microtubule attachments (for reviews, see Ruchaud *et al.* 2007; Lampson and Cheeseman 2011). Interestingly, two studies suggested that Aurora B also promotes outer kinetochore assembly by phosphorylating Dsn1 (Emanuele *et al.* 2008; Yang *et al.* 2008). However, more recent work challenged these findings and instead concluded that Dsn1 phosphorylation contributes to destabilizing erroneous microtubule attachments (Welburn *et al.* 2010). Therefore, the role of Dsn1 phosphorylation by Aurora B remains unclear.

To elucidate the function of Aurora B-dependent phosphorylation of the budding yeast Dsn1 protein, we mutated the two serine residues in the conserved Aurora B consensus sites (S240 and S250, Figure 1A) to either alanine, which prevents phosphorylation, or aspartic acid, which may mimic phosphorylation. When expressed from the endogenous *DSN1* promoter, the single site mutants (*dsn1-S240A* and *dsn1-S250A*) supported growth in the absence of wild-type (WT) *DSN1* (Westermann *et al.* 2003 and data not shown). However, the *dsn1-S240A, S250A* double mutant failed to complement *dsn1*Δ, suggesting that phosphorylation of at least one of these sites is essential in budding yeast (Akiyoshi *et al.* 2013 and Figure 1B). In contrast, the phosphomimic mutant (*dsn1-S240D, S250D*) is viable.

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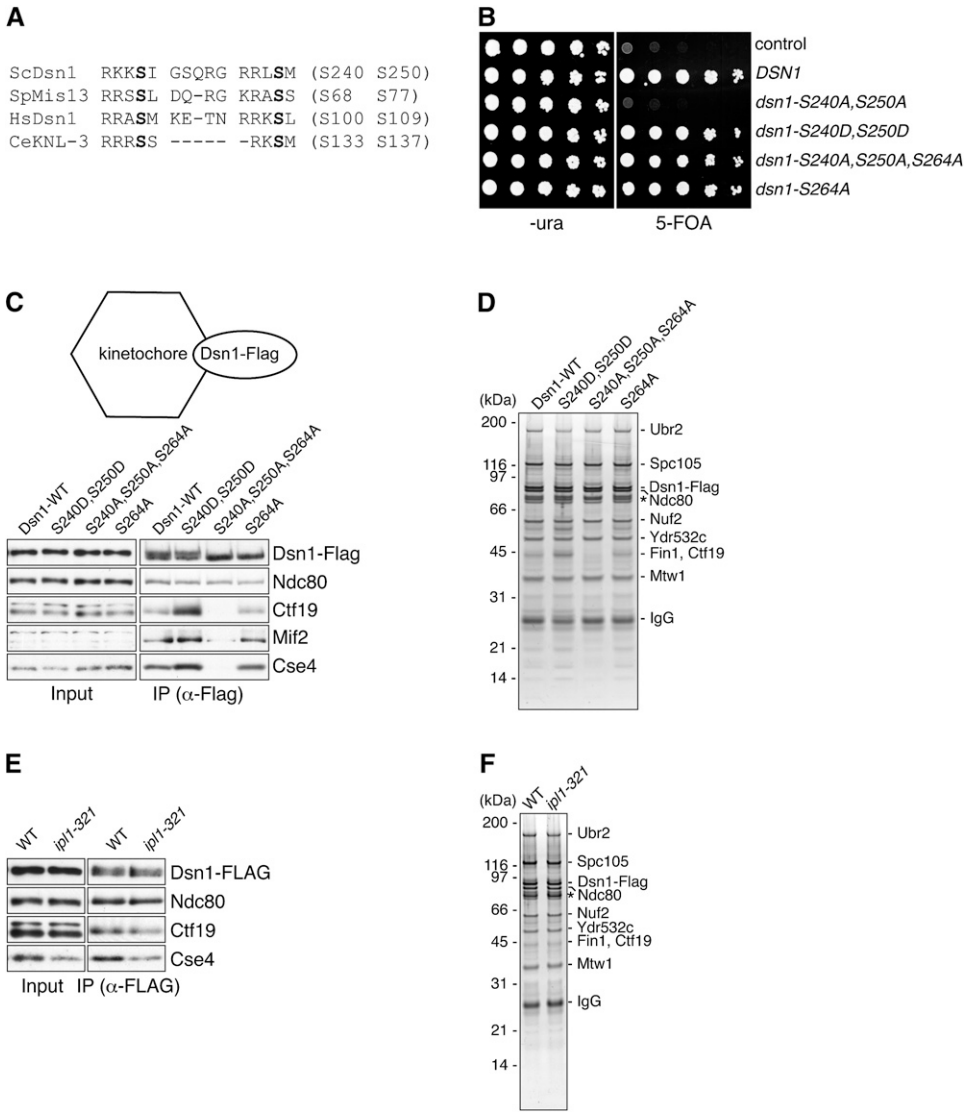

Figure 1 The Dsn1-S240A, S250A mutant that lacks Ipl1 phosphorylation sites is inviable and has defective interactions with inner kinetochore proteins. (A) Dsn1 contains two Ipl1/Aurora B phosphorylation motifs (R/K-R/K-X-pS/T-V/I/L/X). Alignment shows the conservation of Ser residues (shown in boldface type). (B) *Dsn1-S240A*, *S250A* cells are inviable and rescued by the additional mutation of *S264A*. Serial dilutions (fivefold) of *dsn1*Δ cells containing *DSN1* on a *URA3*, CEN vector and the indicated integrated point mutants (SBY2318, SBY5948, SBY5949, SBY5950, SBY5952, and SBY6072) were plated on −ura and 5-FOA plates. Cells that need to maintain the *URA3*, *DSN1* vector for viability are sensitive to 5-FOA. (C and D) Dsn1-S240A, S250A, S264A shows a weakened interaction with the inner-kinetochore components Cse4/CENP-A, Mif2/CENP-C, and Ctf19. Dsn1-Flag proteins containing the indicated mutations (SBY7902, SBY8037, SBY7904, and SBY8123) were immunoprecipitated with anti-Flag antibodies and analyzed by SDS–PAGE followed by immunoblotting against representative kinetochore proteins (C) or silver staining (D) as previously described (Akiyoshi *et al.* 2010). Note that Mif2/CENP-C is not detectable in lysate and the asterisk (*) indicates proteins that non-specifically co-purify with all Flag-tagged proteins in D. (E and F). Dsn1-Flag was immunoprecipitated from lysates prepared from WT (SBY7441) or *ipl1-321* (SBY8120) strains shifted to 37° for 3 hr and analyzed by SDS–PAGE followed by immunoblotting against representative kinetochore proteins (E) or silver staining (F). Strains are listed in supporting information, Table S1.

Dsn1 is also phosphorylated at three Cdk1 consensus sites (T12, S69, and S264), among which only S264 is conserved within the *Saccharomyces* lineage (Akiyoshi and Biggins 2010). While studying Dsn1 phosphorylation, we found that the *dsn1-S240A*, *S250A*, *S264A* triple mutant supports growth in the absence of wild-type *DSN1* (Figure 1B). We previously found that the Dsn1-S240A, S250A mutant protein is targeted for degradation and the addition of the S264A mutation restores protein levels, suggesting that Cdk1 phosphorylation is involved in regulating Dsn1 protein levels (Akiyoshi *et al.* 2013 and data not shown).

Given the controversial role of Aurora B-dependent phosphorylation in regulating Dsn1 (Emanuele *et al.* 2008; Yang *et al.* 2008; Welburn *et al.* 2010), we analyzed kinetochore assembly in the viable Dsn1 phosphomutants and the *ipl1-321* temperature-sensitive mutants using two complementary assays that we established (Akiyoshi *et al.* 2009, 2010). In one technique, kinetochore particles are isolated via the purification of Dsn1, which yields kinetochore particles that contain both inner and outer kinetochore proteins (Akiyoshi *et al.* 2010). However, centromeric DNA and the centromere-binding complex CBF3 do not co-purify, indicating that these particles are not bound to endogenous centromeres after the purification. When we purified Dsn1-Flag from strains containing either wild-type Dsn1 or the Dsn1 phosphomutants that can support viability, there was an equivalent association of the outer KMN network components in all strains (Figure 1, C and D). However, the amounts of co-purifying inner kinetochore components (Ctf19, Mif2/CENP-C, and Cse4/CENP-A) were greatly reduced in the Dsn1-S240A, S250A, S264A mutant strain (Figure 1, C and D). In contrast, the level of inner kinetochore proteins that co-purified with the phosphomimic Dsn1-

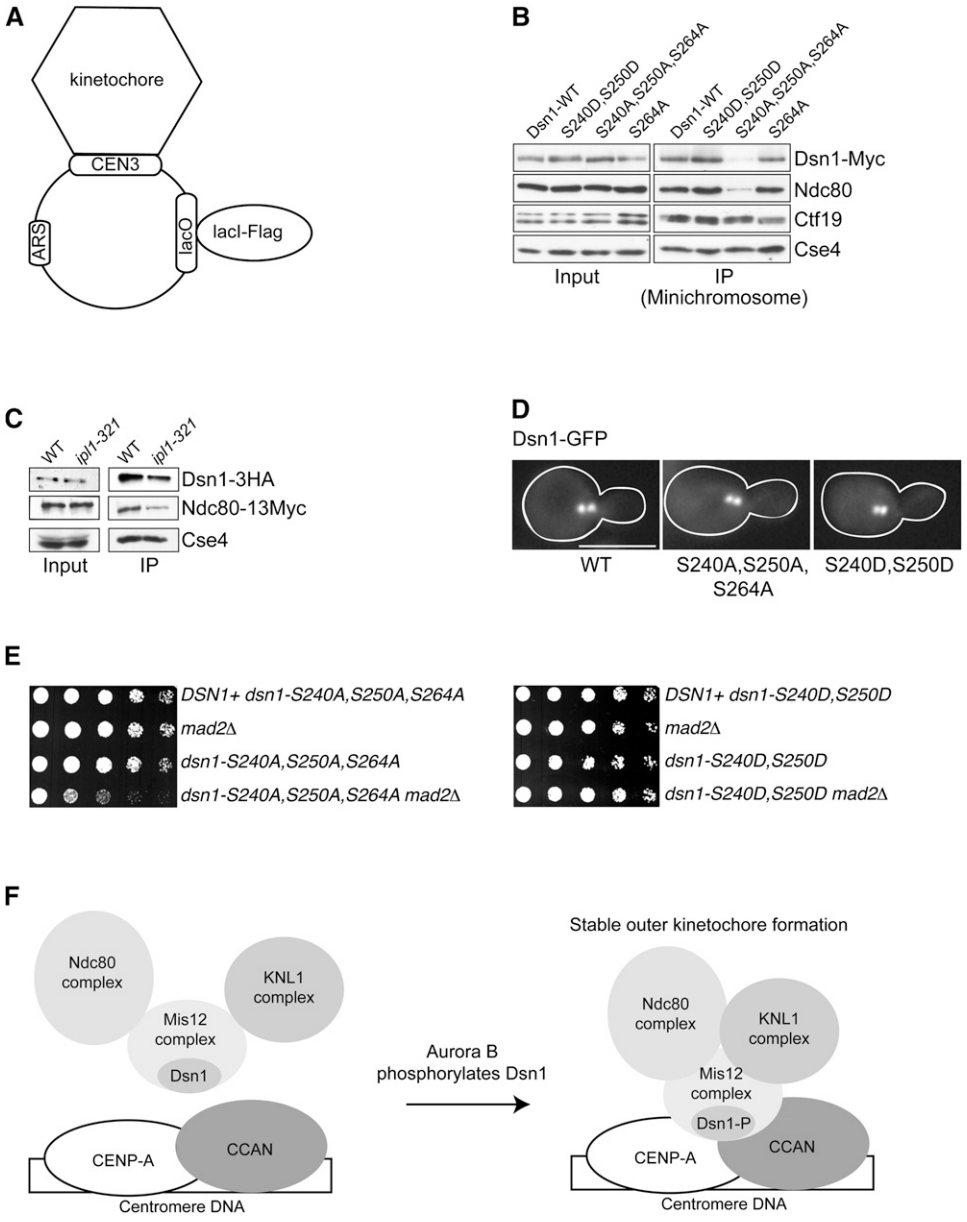

Figure 2 Outer kinetochore proteins have a weakened association with inner kinetochores in *dsn1-S240A*, *S250A*, *S264A* cells. (A) Centromeric minichromosomes containing kinetochores were purified from strains via a LacI-Flag protein that recognizes lacO repeats in the minichromosomes as previously described (Akiyoshi *et al.* 2009). (B) Minichromosomes were purified from strains containing the indicated Dsn1 mutants (SBY8338, SBY8339, SBY8340, and SBY8341) and analyzed via immunoblot with the indicated antibodies. (C) Minichromosomes were purified from WT and *ipl1-321* strains (SBY7824 and SBY7823) that had been shifted to 37° for 3 hr and analyzed via immunoblot with the indicated antibodies. (D) All cells expressing GFP-tagged Dsn1 (SBY7774), Dsn1-S240A, S250A, S264A (SBY7776), and Dsn1-S240D, S250D (SBY7775) mutants show a normal bilobed kinetochore distribution *in vivo*. Bar, 5 μm. (E) *Dsn1-S240A*, *S250A*, *S264A* cells, but not *dsn1-S240D*, *S250D* cells, exhibit a genetic interaction with the spindle checkpoint mutant *mad2Δ*. Serial dilutions (fivefold) of *dsn1-S240A*, *S250A*, *S264A* (left; SBY5952, SBY11599, SBY5956, and SBY11601, respectively) or *dsn1-S240D*, *S250D* cells (right; SBY5950, SBY11599, SBY5954, and SBY11600, respectively) with or without *mad2Δ* were plated on YPD at 30°. (F) Model for the role of Dsn1 phosphorylation by the Aurora B kinase. Strains are listed in Table S1.

S240D, S250D mutant was increased (Figure 1C). Together, these data suggest that phosphorylation of Dsn1 promotes its association with inner kinetochore proteins. Importantly, there were no detectable defects in the single Dsn1-S264A strain, suggesting that the effects we observe in the Dsn1-S240A, S250A, S264A strain are largely due to a lack of Aurora B phosphorylation. Consistent with this, we also found that lower amounts of the inner kinetochore proteins Ctf19 and Cse4 co-purified with Dsn1 from *ipl1-321* mutant cells (Figure 1E). The levels of the outer kinetochore components were normal based on immunoblotting of Dsn1 and Ndc80 as well as silver-stained PAGE stoichiometry estimates (Figure 1, E and F).

In a complementary approach, we utilized a centromeric minichromosome purification method where only centromere-bound kinetochore proteins are co-purified (Figure 2A and Akiyoshi *et al.* 2009). We purified minichromosomes from cells expressing Myc-tagged Dsn1 mutants and examined the amount of kinetochore proteins that remain associated with centromeric DNA by immunoblotting (Figure 2B). Although normal amounts of inner kinetochore proteins were associated with the minichromosome in all strains, the levels of co-purifying outer kinetochore proteins (Dsn1 and Ndc80) were reduced in the *dsn1-S240A*, *S250A*, *S264A* mutant compared to controls (Figure 2B). The single *dsn1-S264A* mutation did not exhibit any obvious defect in kinetochore assembly (Figures 1C and 2B), suggesting that the observed defects of *dsn1-S240A*, *S250A*, *S264A* are largely attributable to *dsn1-S240A*, *S250A*. Consistent with this, the levels of Cse4 that co-purify with minichromosomes from an *ipl1-321* temperature-sensitive mutant are normal, but there are reduced levels of the Ndc80 and Dsn1

kinetochore components (Figure 2C). Our data suggest that the amount of outer kinetochore proteins that assemble onto a centromere in cells defective for Aurora B-mediated phosphorylation of Dsn1 might be low. Alternatively, the association between Dsn1 and inner kinetochore components may be weakened in the absence of Aurora B phosphorylation, leading to loss of their interaction during purification. Because the phosphodeficient Dsn1 mutant properly localizes to kinetochores *in vivo* (Figure 2D) and the mutant supports viability (Figure 1B), we favor the latter possibility. To determine whether there may be subtle structural defects *in vivo*, we tested whether the Dsn1 phosphomutants exhibit genetic interactions with mutants in the spindle checkpoint. The *dsn1-S240A*, *S250A*, *S264A* mutant strain exhibited genetic interactions with a deletion in the *MAD2* spindle checkpoint gene, but the *dsn1-S240D*, *S250D* did not (Figure 2E). These data support the model that phosphorylation of Dsn1 by Aurora B promotes outer kinetochore assembly by increasing its affinity toward inner kinetochores (Figure 2F).

The evolutionary conserved Ipl1/Aurora kinase performs various functions throughout mitosis by phosphorylating multiple substrates. It is therefore critical to understand the molecular basis of its action. Although Dsn1 is an Aurora B substrate, the functional relevance of its phosphorylation remains controversial (Westermann *et al.* 2003; Emanuele *et al.* 2008; Yang *et al.* 2008; Welburn *et al.* 2010). Using the genetically tractable budding yeast, our analyses showed that the phosphodeficient Dsn1 mutant has a reduced affinity for inner kinetochore proteins. These results are consistent with previous findings suggesting that Dsn1 phosphorylation by Aurora B is important for outer kinetochore assembly (Emanuele *et al.* 2008; Yang *et al.* 2008). We previously found that the Dsn1-S240A, S250A mutant is targeted for degradation by the Mub1/Ubr2 ubiquitin ligase complex (Akiyoshi *et al.* 2013), suggesting that defects in kinetochore assembly may be monitored by a quality control system. However, in contrast to these findings, kinetochore assembly appears intact in the budding yeast *ipl1-321* temperature-sensitive mutant (Pinsky *et al.* 2006) as well as in Aurora B depleted/inactivated mammalian cells (*e.g.*, Ditchfield *et al.* 2003; Hauf *et al.* 2003). To reconcile these differences, we propose that a minimal level of Aurora B activity is sufficient to phosphorylate Dsn1 and promote kinetochore assembly, whereas higher activity is required to fulfill its other functions (*e.g.*, error correction, spindle assembly, and cytokinesis) (Norden *et al.* 2006; Xu *et al.* 2009). Consistent with this idea, we previously showed that spindle assembly requires higher levels of Ipl1 kinase activity than other Aurora-dependent functions (Kotwaliwale *et al.* 2007). In addition, histone H3 Ser10, a well-established Aurora B substrate, often remains phosphorylated upon Aurora B inactivation (*e.g.*, Kiyomitsu *et al.* 2010). We also note that budding yeast shows differential requirements in the level of Cdk1 needed to complete various cell cycle events (Bishop *et al.* 2000). In the future, it will therefore be important to determine whether the difference is due to altered levels of kinase activity, as well as to understand the underlying basis of the requirement for differential kinase activity. It will also be critical to understand how Dsn1 phosphorylation promotes the interaction between the inner and outer kinetochore to fully understand how kinetochores are assembled and maintained, thus ensuring genomic stability.

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Literature Cited

Akiyoshi, B., and S. Biggins, 2010 Cdc14-dependent dephosphorylation of a kinetochore protein prior to anaphase in *Saccharomyces cerevisiae*. Genetics 186: 1487–1491.

Akiyoshi, B., C. R. Nelson, J. A. Ranish, and S. Biggins, 2009 Quantitative proteomic analysis of purified yeast kinetochores identifies a PP1 regulatory subunit. Genes Dev. 23: 2887–2899.

Akiyoshi, B., K. K. Sarangapani, A. F. Powers, C. R. Nelson, S. L. Reichow *et al.*, 2010 Tension directly stabilizes reconstituted kinetochore-microtubule attachments. Nature 468: 576–579.

Akiyoshi, B., C. R. Nelson, N. Duggan, S. Ceto, J. A. Ranish *et al.*, 2013 The Mub1/Ubr2 ubiquitin ligase complex regulates the conserved Dsn1 kinetochore protein. PLoS Genet. 9: e1003216.

Bishop, A. C., J. A. Ubersax, D. T. Petsch, D. P. Matheos, N. S. Gray *et al.*, 2000 A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature 407: 395–401.

Bloom, K., and A. Joglekar, 2010 Towards building a chromosome segregation machine. Nature 463: 446–456.

Buscaino, A., R. Allshire, and A. Pidoux, 2010 Building centromeres: home sweet home or a nomadic existence? Curr. Opin. Genet. Dev. 20: 118–126.

Carroll, C. W., K. J. Milks, and A. F. Straight, 2010 Dual recognition of CENP-A nucleosomes is required for centromere assembly. J. Cell Biol. 189: 1143–1155.

Cheeseman, I. M., and A. Desai, 2008 Molecular architecture of the kinetochore-microtubule interface. Nat. Rev. Mol. Cell Biol. 9: 33–46.

Cheeseman, I. M., J. S. Chappie, E. M. Wilson-Kubalek, and A. Desai, 2006 The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 127: 983–997.

Davies, A. E., and K. B. Kaplan, 2010 Hsp90-Sgt1 and Skp1 target human Mis12 complexes to ensure efficient formation of kinetochore-microtubule binding sites. J. Cell Biol. 189: 261–274.

Ditchfield, C., V. L. Johnson, A. Tighe, R. Ellston, C. Haworth *et al.*, 2003 Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. J. Cell Biol. 161: 267–280.

Emanuele, M. J., W. Lan, M. Jwa, S. A. Miller, C. S. Chan *et al.*, 2008 Aurora B kinase and protein phosphatase 1 have opposing roles in modulating kinetochore assembly. J. Cell Biol. 181: 241–254.

Gascoigne, K. E., and I. M. Cheeseman, 2011 Kinetochore assembly: if you build it, they will come. Curr. Opin. Cell Biol. 23: 102–108.

Gonen, S., B. Akiyoshi, M. G. Iadanza, D. Shi, N. Duggan *et al.*, 2012 The structure of purified kinetochores reveals multiple

microtubule-attachment sites. Nat. Struct. Mol. Biol. 19: 925–929.

Hauf, S., R. W. Cole, S. LaTerra, C. Zimmer, G. Schnapp *et al.*, 2003 The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. J. Cell Biol. 161: 281–294.

Holland, A. J., and D. W. Cleveland, 2012 Losing balance: the origin and impact of aneuploidy in cancer. EMBO Rep. 13: 501–514.

Joglekar, A. P., D. C. Bouck, J. N. Molk, K. S. Bloom, and E. D. Salmon, 2006 Molecular architecture of a kinetochore-microtubule attachment site. Nat. Cell Biol. 8: 581–585.

Kiyomitsu, T., O. Iwasaki, C. Obuse, and M. Yanagida, 2010 Inner centromere formation requires hMis14, a trident kinetochore protein that specifically recruits HP1 to human chromosomes. J. Cell Biol. 188: 791–807.

Kline, S. L., I. M. Cheeseman, T. Hori, T. Fukagawa, and A. Desai, 2006 The human Mis12 complex is required for kinetochore assembly and proper chromosome segregation. J. Cell Biol. 173: 9–17.

Kotwaliwale, C. V., S. B. Frei, B. M. Stern, and S. Biggins, 2007 A pathway containing the Ipl1/aurora protein kinase and the spindle midzone protein Ase1 regulates yeast spindle assembly. Dev. Cell 13: 433–445.

Lampson, M. A., and I. M. Cheeseman, 2011 Sensing centromere tension: Aurora B and the regulation of kinetochore function. Trends Cell Biol. 21: 133–140.

Lawrimore, J., K. S. Bloom, and E. D. Salmon, 2011 Point centromeres contain more than a single centromere-specific Cse4 (CENP-A) nucleosome. J. Cell Biol. 195: 573–582.

Malvezzi, F., G. Litos, A. Schleiffer, A. Heuck, K. Mechtler *et al.*, 2013 A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors. EMBO J. 32: 409–423.

Maskell, D. P., X. W. Hu, and M. R. Singleton, 2010 Molecular architecture and assembly of the yeast kinetochore MIND complex. J. Cell Biol. 190: 823–834.

Norden, C., M. Mendoza, J. Dobbelaere, C. V. Kotwaliwale, S. Biggins *et al.*, 2006 The NoCut pathway links completion of cytokinesis to spindle midzone function to prevent chromosome breakage. Cell 125: 85–98.

Okada, M., I. M. Cheeseman, T. Hori, K. Okawa, I. X. McLeod *et al.*, 2006 The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. Nat. Cell Biol. 8: 446–457.

Perpelescu, M., and T. Fukagawa, 2011 The ABCs of CENPs. Chromosoma 120: 425–446.

Petrovic, A., S. Pasqualato, P. Dube, V. Krenn, S. Santaguida *et al.*, 2010 The MIS12 complex is a protein interaction hub for outer kinetochore assembly. J. Cell Biol. 190: 835–852.

Pinsky, B. A., C. Kung, K. M. Shokat, and S. Biggins, 2006 The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. Nat. Cell Biol. 8: 78–83.

Powers, A. F., A. D. Franck, D. R. Gestaut, J. Cooper, B. Gracyzk *et al.*, 2009 The Ndc80 kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion. Cell 136: 865–875.

Przewloka, M. R., Z. Venkei, V. M. Bolanos-Garcia, J. Debski, M. Dadlez *et al.*, 2011 CENP-C is a structural platform for kinetochore assembly. Curr. Biol. 21: 399–405.

Ruchaud, S., M. Carmena, and W. C. Earnshaw, 2007 Chromosomal passengers: conducting cell division. Nat. Rev. Mol. Cell Biol. 8: 798–812.

Screpanti, E., A. De Antoni, G. M. Alushin, A. Petrovic, T. Melis *et al.*, 2011 Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. Curr. Biol. 21: 391–398.

Tanaka, K., H. L. Chang, A. Kagami, and Y. Watanabe, 2009 CENP-C functions as a scaffold for effectors with essential kinetochore functions in mitosis and meiosis. Dev. Cell 17: 334–343.

Welburn, J. P., M. Vleugel, D. Liu, J. R. Yates, 3rd, M. A. Lampson *et al.*, 2010 Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. Mol. Cell 38: 383–392.

Westermann, S., I. M. Cheeseman, S. Anderson, J. R. Yates, 3rd, D. G. Drubin *et al.*, 2003 Architecture of the budding yeast kinetochore reveals a conserved molecular core. J. Cell Biol. 163: 215–222.

Westermann, S., D. G. Drubin, and G. Barnes, 2007 Structures and functions of yeast kinetochore complexes. Annu. Rev. Biochem. 76: 563–591.

Xu, Z., H. Ogawa, P. Vagnarelli, J. H. Bergmann, D. F. Hudson *et al.*, 2009 INCENP-aurora B interactions modulate kinase activity and chromosome passenger complex localization. J. Cell Biol. 187: 637–653.

Yang, Y., F. Wu, T. Ward, F. Yan, Q. Wu *et al.*, 2008 Phosphorylation of HsMis13 by Aurora B kinase is essential for assembly of functional kinetochore. J. Biol. Chem. 283: 26726–26736.

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Table S1 Yeast strains used in this study. All strains are isogenic with the W303 background and were generated by standard procedures. Plasmids are indicated in brackets.

Strain	Genotype
SBY2318	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3]*
SBY5948	*MAT**a** ura3-1 leu2,3-112 his3-11::DSN1::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1097; DSN1, HIS3]*
SBY5949	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1-S240A,S250A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1099; dsn1-S240A,S250A, HIS3]*
SBY5950	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1-S240D,S250D::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1104; dsn1-S240D,S250D, HIS3]*
SBY5952	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1-S240A,S250A,S264A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB1108; dsn1-S240A,S250A,S264A, HIS3]*
SBY5954	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1-S240D,S250D::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB1104; dsn1-S240D,S250D, HIS3]*
SBY5956	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1-S240A,S250A,S264A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB1108; dsn1-S240A,S250A,S264A, HIS3]*
SBY6072	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1- S264A::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ [pSB624; DSN1, CEN, URA3][pSB944; dsn1-S264A, HIS3]*
SBY7441	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2Δ can1-100 bar1Δ DSN1-3FLAG::KanMX*
SBY7774	*MAT**a** ura3-1 leu2,3-112 his3-11::DSN1-3GFP::HIS3 trp1-1 ade2-1 LYS2*

can1-100 dsn1::KanMX bar1Δ

SBY7775 *MAT**a** ura3-1 leu2,3-112 his3-11::DSN1-S240D,S250D-3GFP::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ*

SBY7776 *MAT**a** ura3-1 leu2,3-112 his3-11::DSN1-S240A,S250A,S264A-3GFP::HIS3 trp1-1 ade2-1 LYS2 can1-100 dsn1::KanMX bar1Δ*

SBY7823 *MAT**a** ura3-1::pCMV-LacI-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LYS2 BAR1 NDC80-13myc::KAN ipl1-321 DSN1-3HA::HIS3 [WT CEN3 Minichromosome, TRP1]*

SBY7824 *MAT**a** ura3-1::pCMV-LacI-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 can1-100 LYS2 BAR1 NDC80-13myc::KAN DSN1-3HA::HIS3 [WT CEN3 Minichromosome, TRP1]*

SBY7902 *MAT**a** ura3-1::DSN1-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY7904 *MAT**a** ura3-1::DSN1-S240A,S250A,S264A-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY8037 *MAT**a** ura3-1::DSN1-S240D,S250D-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY8120 *MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2Δ can1-100 bar1Δ ipl1-321 DSN1-3FLAG::KanMX*

SBY8123 *MAT**a** ura3-1::DSN1-S264A-3FLAG::URA3 leu2,3-112 his3-11 trp1-1 ade2-1 LYS2 can1-100 bar1Δ dsn1::KanMX*

SBY8338 *MAT**a** ura3-1::DSN1-12MYC::URA3 leu2,3-112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]*

SBY8339 *MAT**a** ura3-1::DSN1-S240D,S250D-12MYC::URA3 leu2,3-112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]*

SBY8340 *MAT**a** ura3-1::DSN1-S240A,S250A,S264A-12MYC::URA3 leu2,3-*

	112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]
SBY8341	*MAT**a** ura3-1::DSN1-S264A-12MYC::URA3 leu2,3-112::LacI-FLAG::LEU2 his3-11 trp1-1 ade2-1 can1-100 LYS2 bar1 dsn1::KanMX [pSB963, CEN3, 8lacO, TRP1]*
SBY11599	*MAT**a** ura3-1 leu2,3-112 his3-11 trp1-1 ade2-1 lys2Δ can1-100 mad2::URA3 bar1Δ*
SBY11600	*MAT**a** ura3-1 leu2,3-112 his3-11::dsn1-S240D,S250D::HIS3 trp1-1 ade2-1 lys2Δ can1-100 mad2::URA3 bar1Δ dsn1::KanMX bar1Δ [pSB1104; dsn1-S240D,S250D, HIS3]*
SBY11601	*MAT**a** ura3-1 leu2,3-112 his3-11:: dsn1-S240A,S250A,S264A::HIS3 trp1-1 ade2-1 lys2Δ can1-100 mad2::URA3 dsn1::KanMX bar1Δ [pSB1108; dsn1-S240A,S250A,S264A, HIS3]*

Media and genetic and microbial techniques were essentially as described (Rose et al., 1990). Phospho-mutants were made by Quickchange site-directed mutagenesis (Stratagene) and are described in (AKIYOSHI *et al.* 2013).

Supplemental Literature Cited

AKIYOSHI, B., C. R. NELSON, N. DUGGAN, S. CETO, J. A. RANISH *et al.*, 2013 The Mub1/Ubr2 ubiquitin ligase complex regulates the conserved Dsn1 kinetochore protein. PLoS Genet **9:** e1003216.