Current Biology, Vol. 15, 1–7, June 7, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.05.020

A Small-Molecule Inhibitor of Mps1 Blocks the Spindle-Checkpoint Response to a Lack of Tension on Mitotic Chromosomes

Russell K. Dorer,[1,2,4,7] Sheng Zhong,[6,8] John A. Tallarico,[4] Wing Hung Wong,[3,6,8] Timothy J. Mitchison,[4,5] and Andrew W. Murray[1,2,*]
[1]Department of Molecular and Cellular Biology
[2]Bauer Center for Genomics Research
[3]Department of Statistics
Harvard University
Cambridge, Massachusetts 02138
[4]Institute of Chemistry and Cell Biology
[5]Department of Systems Biology
250 Longwood Avenue
Harvard Medical School
Boston, Massachusetts 02115
[6]Department of Biostatistics
Harvard School of Public Health
Boston, Massachusetts 02115

Summary

The spindle checkpoint prevents chromosome loss by preventing chromosome segregation in cells with improperly attached chromosomes [1–3]. The checkpoint senses defects in the attachment of chromosomes to the mitotic spindle [4] and the tension exerted on chromosomes by spindle forces in mitosis [5–7]. Because many cancers have defects in chromosome segregation, this checkpoint may be required for survival of tumor cells and may be a target for chemotherapy. We performed a phenotype-based chemical-genetic screen in budding yeast and identified an inhibitor of the spindle checkpoint, called cincreasin. We used a genome-wide collection of yeast gene-deletion strains and traditional genetic and biochemical analysis to show that the target of cincreasin is Mps1, a protein kinase required for checkpoint function [8]. Despite the requirement for Mps1 for sensing both the lack of microtubule attachment and tension at kinetochores, we find concentrations of cincreasin that selectively inhibit the tension-sensitive branch of the spindle checkpoint. At these concentrations, cincreasin causes lethal chromosome missegregation in mutants that display chromosomal instability. Our results demonstrate that Mps1 can be exploited as a target and that inhibiting the tension-sensitive branch of the spindle checkpoint may be a way of selectively killing cancer cells that display chromosomal instability.

Results and Discussion

A Small-Molecule Inhibitor of the Spindle-Checkpoint Delay Caused by Kinetochore Tension Defects

We devised a screen in which molecules that inhibit the spindle checkpoint stimulate cell proliferation. Linear minichromosomes (10–15 kb) segregate poorly in mitosis, activate the spindle checkpoint, and have a much higher loss frequency than their circular counterparts or natural chromosomes [9]. The *CDC28-VF* mutation lies in the critical cyclin-dependent kinase that drives cells into mitosis and extends this checkpoint-dependent delay and prevents colonies from forming on solid medium. We used a strain containing the *CDC28-VF* mutation, a linear minichromosome, and P_{TET}-*CDC20-127*, a dominant, checkpoint-inhibitory allele of *CDC20* (an essential activator of the ubiquitin-dependent proteolysis that initiates chromosome segregation) under the control of the tetracycline promoter [10]. In the absence of doxycycline, *CDC20-127* is expressed and the checkpoint is inactivated, but in the presence of doxycyline, *CDC20-127* is repressed and the checkpoint is restored. Thus, combining P_{TET}-*CDC20-127*, *CDC28-VF*, and a linear minichromosome creates a strain that cannot grow in the presence of doxycycline because the minichromosome activates the spindle checkpoint. This strain allowed us to identify checkpoint inhibitors that enable faster growth in the presence of doxycycline. Because we screened for increased cell proliferation, our screen selected for a minimal level of specificity: Compounds that strongly inhibit any of the more than 1100 yeast proteins that are essential for viability could not be recovered.

We screened 140,000 small molecules and identified five compounds (from the Chembridge DIVERSet E) that permitted proliferation to the same extent as deletion of *MAD2*, a known spindle-checkpoint gene. We resynthesized these compounds (see Supplemental Experimental Procedures), tested intermediates in the above-described minichromosome assay, and found a single active compound common to all five reagents (Figure 1A); we named this compound "cincreasin" (chromosome *in*stability *increasin*g compound). Related compounds were less active (see Figure S1 in the Supplemental Data available with this article online). A dose-response assay showed that cincreasin activates proliferation in the minichromosome assay at 30–40 μM, about 10-fold lower than the IC_{50} for viability (Figure S2).

We tested whether cincreasin overcomes the spindle-checkpoint arrest caused by disrupting the microtubule-kinetochore attachments with the microtubule-depolymerizing drug benomyl. The spindle checkpoint arrests wild-type cells treated with 60 μg/ml benomyl in mitosis for several hours, and viability is maintained [1, 2]. In this situation, cells deleted for *BUB2* delay in mitosis at the spindle checkpoint but gradually lose viability because they eventually bud despite the improper placement of the spindle [11–13]. Cells deleted for both *BUB2* and *MAD2* do not delay when treated with microtubule-depolymerizing drugs and rapidly lose viability. We measured the viability and rate of rebudding of *bub2Δ* in 60 μg/ml benomyl plus DMSO, *bub2Δ* in 60 μg/ml benomyl plus 150 μM cincreasin, and *bub2Δ mad2Δ* in 60 μg/ml benomyl plus DMSO. We found no effect of cincreasin on either viability or rebudding in

*Correspondence: amurray@mcb.harvard.edu
[7]Present address: Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115.
[8]Present address: Department of Statistics, Stanford University, Sequoia Hall, 390 Serra Mall, Stanford, California 94305.

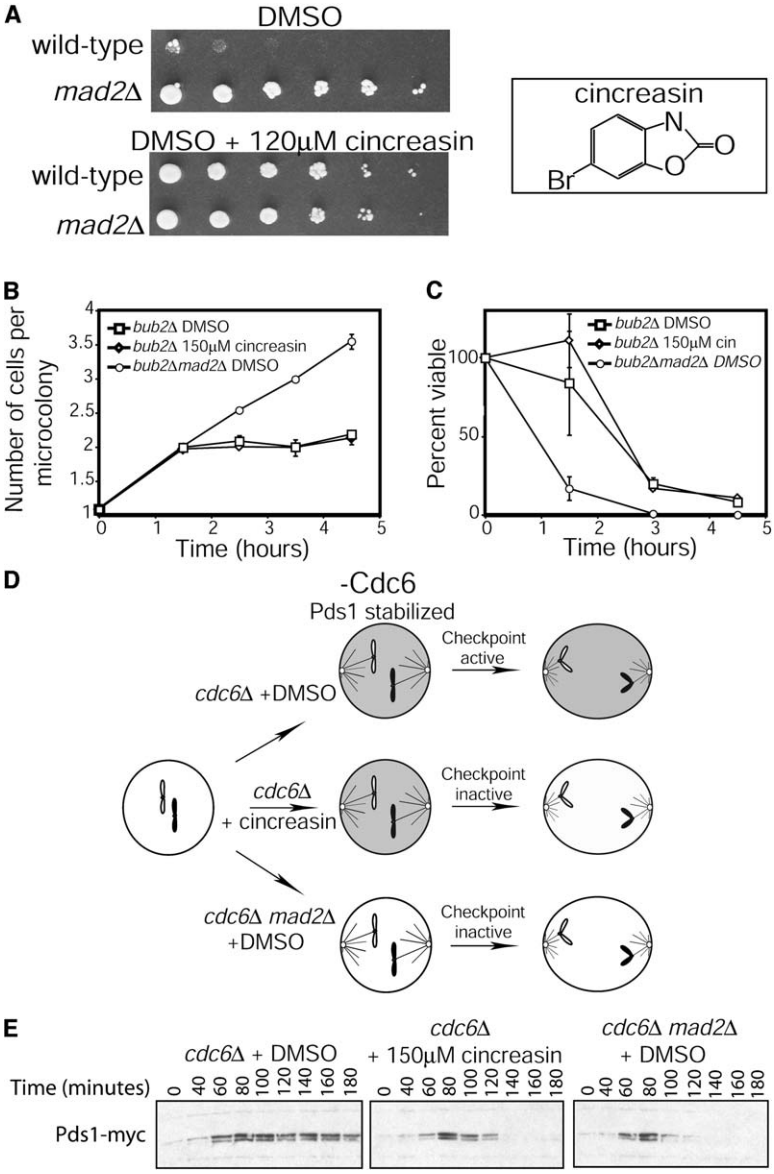

Figure 1. Cincreasin Inhibits the Tension-Dependent Spindle Checkpoint but Does Not Inhibit the Attachment-Sensitive Checkpoint

(A) Cincreasin bypasses the growth delay caused by linear minichromosomes. Five-fold serial dilutions of YBS151 (*CDC28VF tet-CDC20-127+ SLC::LEU2*) were spotted on minimal plates containing 0.1% DMSO and the indicated concentration of *cincreasin* and then grown for 2 days at 30°C and photographed.

(B) and (C) The mean plus standard deviation of the viability and rate of rebudding of the indicated *bub2Δ* strains treated with the microtubule-depolymerizing drug benomyl (60 μg/ml). No effect of cincreasin on viability or rebudding was found.

(D) Experimental design to evaluate the checkpoint response to tension defects (modified from [7]). Cdc6-depleted cells go through mitosis without replicating their chromosomes. Kinetochores that are not under tension activate the spindle checkpoint and stabilize Pds1 (gray). Inactivation of the checkpoint by deletion of *MAD2* or treatment with cincreasin allows degradation of Pds1.

(E) Pds1p levels were monitored during the cell cycle (as in [7]) in cells that were depleted of Cdc6 (YBS420) and were compared to the levels in Cdc6-depleted cells that also lacked Mad2 (YBS514); see Supplemental Experimental Procedures for details.

benomyl, indicating that cincreasin is unable to inhibit the attachment-sensitive branch of the spindle checkpoint (Figures 1B and C).

Tension is generated on kinetochores during mitosis when the poleward microtubule-dependent forces exerted on kinetochores are opposed by the linkage between sister chromatids [5–7]. Cells depleted for the replication protein Cdc6 do not replicate their DNA, but they still proceed through mitosis. However, tension cannot be generated because the chromatids lack sisters, and the spindle checkpoint is activated, stabilizing Pds1p, a protein that inhibits sister-chromatid segregation and is a target of Cdc20-dependent ubiquitination [7, 14]. We evaluated the effects of cincreasin on Pds1p degradation in cells that lack Cdc6 (*cdc6Δ GAL-CDC6*) compared to in Cdc6-depleted cells that lack Mad2 (*cdc6Δ GAL-CDC6 mad2Δ,* Figures 1D and 1E). Pds1p levels were stabilized in control DMSO-treated cells containing unreplicated DNA but not in cells treated with 150 μM cincreasin or in *mad2Δ* cells, indicating that cincreasin keeps the spindle checkpoint from delaying the exit from mitosis in cells whose kinetochores are not under tension.

Cincreasin Increases the Chromosome Loss Rate in Wild-Type Cells and Checkpoint Mutants

Because cincreasin perturbs the response to chromosomes that are not under tension, we measured the chromosome loss rate in wild-type yeast cells treated with cincreasin by determining the loss rate of a nonessential test chromosome via a colony-color assay [15, 16]. The test strain harbors the *ade2-101* (ochre) mutation and a test chromosome bearing the *SUP11* gene (an ochre-suppressing tRNA), which is lost 100 times more frequently than normal chromosomes. Losing the test chromosome makes the cells red instead of white.

If this loss occurs in the first division, the resulting colony is half red and half white, and the rate of chromosome missegregation is calculated by dividing the number of half-red colonies by the total number of colonies containing the test chromosome (as in [16]). We found that cincreasin increases the chromosome loss rate of wild-type W303 cells in a dose-dependent manner (Figure 2A), indicating that cincreasin strongly affects the fidelity of chromosome segregation in otherwise-unperturbed mitoses.

To determine whether the effect of cincreasin on chromosome segregation can be explained solely by inhibition of the spindle checkpoint, we measured the loss rate in cells deleted for several checkpoint genes and treated with cincreasin. We found that cincreasin dramatically increased the loss rate in *mad1Δ*, *mad2Δ*, and *mad3Δ* cells (10-fold, 20-fold, and 15-fold at 60, 60, and 120 μM, respectively; Figure 2B); the loss rates of *mad1Δ* and *mad2Δ* mutants at 120 μM cincreasin were too high to measure accurately. Because the effects of cincreasin on these checkpoint mutants are so severe, we conclude that cincreasin must inhibit other aspects of chromosome segregation in addition to the spindle checkpoint and that the target of cincreasin cannot solely be Mad1, Mad2, or Mad3.

A Genome-Wide Genetic Screen Reveals a Unique Chemogenetic Sensitivity Profile for Cincreasin

Cincreasin might interfere with two aspects of mitosis, the tension-sensitive branch of the spindle checkpoint, and some other mitotic process. To investigate this issue, we performed a genome-wide screen for deletion mutants sensitive to cincreasin. We inoculated ~4,700 haploid deletion mutants onto rich medium plus either 200 μM cincreasin or DMSO and found 124 cincreasin-sensitive strains. Of these 124 strains, 71 mutants scored strongly (32; +++) or moderately (39; ++) sensitive to cincreasin (Table S1). Sixteen of the 32 (50%) strong and 7 of the 39 (18%) moderate mutants have previously-reported defects in kinetochore structure and function (*mcm16Δ*, *mcm17Δ*, *mcm21Δ*, *mcm22Δ*, *ctf19Δ*, *ctf3Δ*, *ctf4Δ*, *bim1Δ*, *chl1Δ*, and *sgo1Δ*), microtubule motors (*cin8Δ* and *kar3Δ*), microtubule stability or structure (*gim3Δ*, *gim4Δ*, and *gim5Δ*), sister-chromatid cohesion (*dcc1Δ* and *ctf8Δ*), the spindle checkpoint (*bub1Δ*, *bub3Δ*, and *sgo1Δ*), or other aspects of mitosis (*cdh1Δ*, *rts1Δ*, *pac10Δ*, and *cik1Δ*) (reviewed in [17]). Representative mutants are shown (Figure 2C). The strong sensitivity of *bub1Δ* and *bub3Δ* mutants excludes Bub1 and Bub3 as the sole targets of cincreasin. Some of the remaining deletion mutants (i.e., *VMA8*, *VMA22*, *LSM1*, *YGL072C*, *YDJ1*, *YEL059W*, and *RVS167*) were recently identified as sensitive to at least four of ten compounds of diverse activities and structure and thus may be multidrug resistant [18]. Nevertheless, when functionally analyzed with the gene ontogeny (GO) term finder (www.yeastgenome.org), no other major cellular-process categories outside of genes involved in chromosome segregation are significantly enriched (data not shown). Moreover, *dam1-11*, *dam1-24*, and *dad1-1* mutants, critical for microtubule-kinetochore interactions [19–21], are very cincreasin-sensitive (data not shown). These data suggest two possibilities. Either the

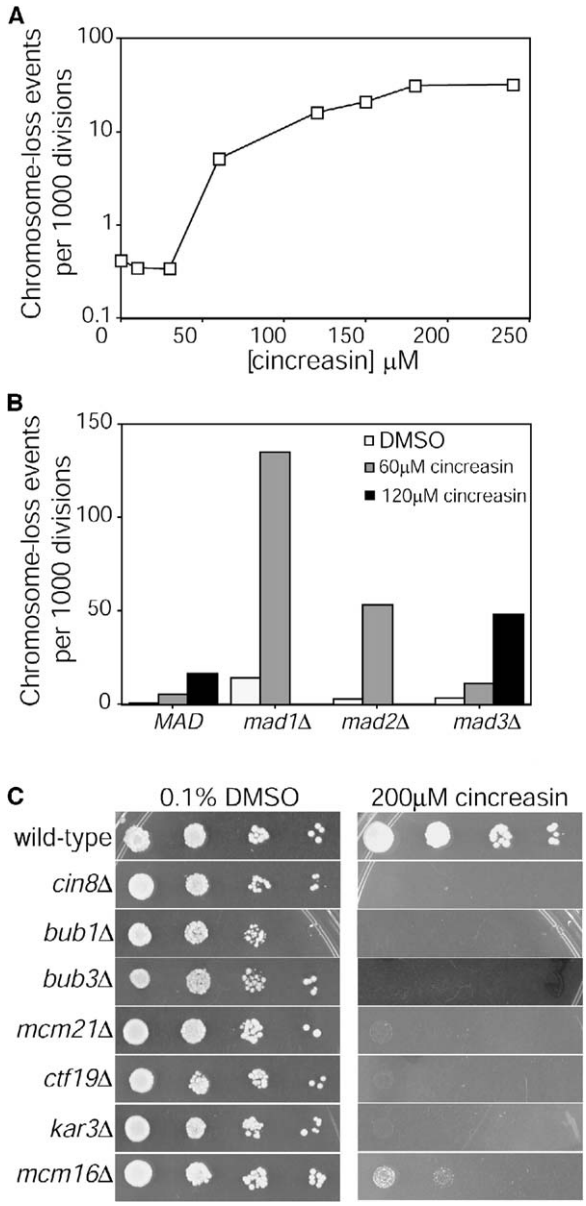

Figure 2. Cincreasin Increases Chromosome Loss in Wild-Type and Checkpoint Mutants

The loss of a nonessential test chromosome was scored with a colony-color assay to measure the effect of cincreasin on chromosome loss rate of wild-type and *mad1Δ*, *mad2Δ*, or *mad3Δ* mutants (see Supplemental Experimental Procedures for details).

(A) Wild-type cells (YMB108) treated with the indicated concentration of cincreasin.

(B) Wild-type (YMB108) and *madΔ* mutants (YMB111, YMB113, and YJR111) treated with DMSO, 60 μM, or 120 μM cincreasin.

(C) Mutants defective in chromosome segregation are extremely sensitive to cincreasin. Ten-fold serial dilutions of the indicated haploid deletion strain (isogenic with the wild-type control [BY4741]) were spotted on YPD (rich media) + 0.1% DMSO or YPD + 0.1% DMSO and 200 μM cincreasin, grown 2 days at 30°C, and photographed. The mutants shown were strongly sensitive to cincreasin (+++, no growth), except *mcm16Δ*, which was moderately sensitive (++, growth of the highest dilution only). In another assay, the IC_{50} for *cin8Δ* (approximately 40 μM) is 10–20-fold lower than for wild-type (data not shown).

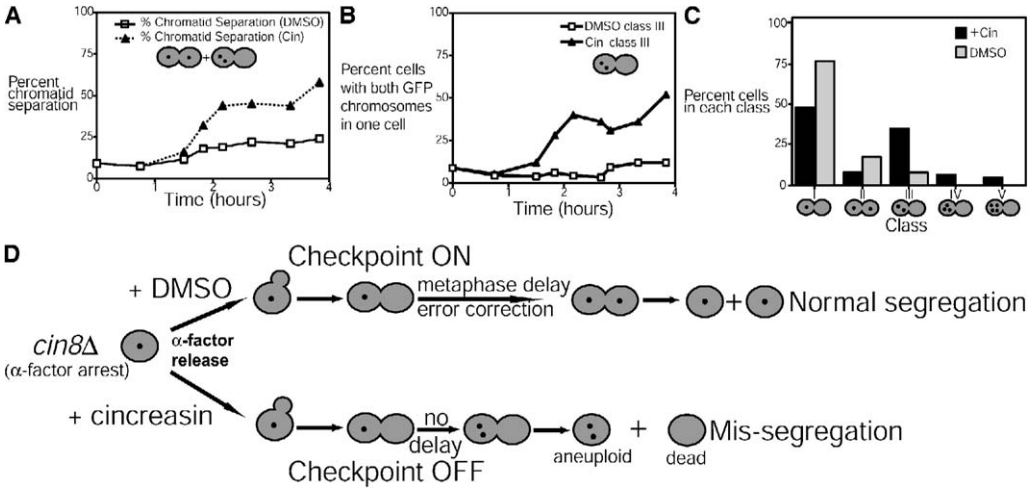

Figure 3. Cincreasin Causes Missegregation of Chromosomes in *cin8⊿* Cells

(A) Haploid *cin8⊿* cells were released from α-factor arrest into media containing either DMSO or 120 μM cincreasin. The percentage of large-budded cells in which any two GFP-labeled sister chromatids separated was counted at the indicated time points. In DMSO, the majority of chromatids do not separate because cells delay in metaphase at the spindle checkpoint.

(B) The percentage of cells in which both GFP-labeled chromosomes missegregated to the same pole.

(C) The percentages of cells with the indicated number of GFP-labeled chromosomes were measured 7.5 hr after release into media containing cincreasin or DMSO (as in [A]).

(D) Schematic interpretation of this experiment. *cin8⊿* cells treated with cincreasin separated their chromosomes without a checkpoint-dependent delay and often missegregated their chromosomes to the same pole, resulting in aneuploidy and death. DMSO-treated cells delayed in metaphase at the checkpoint, corrected errors due to the *cin8⊿* mutation in segregation, and then segregated their chromosomes normally.

sensitivity of every mutant is due to cincreasin effects on the spindle checkpoint, effects that make cells very sensitive to a variety of spindle defects, or the sensitivity of some mutants is due to direct cincreasin effects on spindle structure and function. We favor the latter. Taken together, these data suggest that cincreasin inhibits microtubule and/or kinetochore function in addition to the spindle checkpoint.

Many cincreasin-sensitive mutants affect chromosome segregation and require the spindle checkpoint for survival. For example, *cin8⊿* mutants, which lack a microtubule motor, are synthetically lethal with deletions of *MAD1* or *MAD2* [22]. To test whether the checkpoint causes a metaphase delay in *cin8⊿* mutants and promotes proper chromosome segregation, cells with green fluorescent protein (GFP)-labeled chromosomes [23] were synchronized in G1 with α-factor and released into media containing either DMSO (control) or cincreasin. *cin8⊿* mutants treated with DMSO (control) delayed in metaphase for 2–4 hr and then proceeded through anaphase asynchronously (Figure 3A). However, when treated with cincreasin, *cin8⊿* mutants began to separate sister chromatids 90 min after release from G1 (Figure 3A). In addition, cincreasin caused massive chromosome loss in *cin8⊿* cells (Figures 3A and 3B), which produced many aneuploid cells after 7.5 hr (Figure 3C). Thus the strong cincreasin sensitivity of *cin8⊿* mutants is explained by massive chromosome missegregation (summarized in Figure 3D), suggesting that in *cin8⊿* mutants, some chromosomes are not under tension because both sister kinetochores have attached to the same pole (mono-orientation), thus activating the checkpoint. The high fraction of cells that complete mitosis with both copies of the labeled chromosome in a single daughter cell can be explained in two ways: The absence of Cin8 causes most chromosomes to mono-orient and the checkpoint holds cells in prometaphase while this defect is corrected, or cincreasin increases the probability of mono-orientation in *cin8⊿* cells, as well as inhibiting the checkpoint.

Target Identification with Drug-Induced Haploinsufficiency

In general, it is hard to identify the target of a chemical inhibitor found in phenotype-based screens. In mammalian cells, most approaches are difficult and require a high affinity for the target [24]. In yeast, there is a genetic approach, which takes advantage of the fact that a heterozygous strain deleted for one of the two copies of a drug's target typically expresses less of the target and is hypersensitive to the drug [25–27]. We performed a genome-wide screen of diploid cells containing heterozygous gene deletions to identify potential targets of cincreasin. A simple small molecule like cincreasin may bind with low affinity to many proteins, so we expected to find several potential targets. Because the growth assays are competitive, fitness profiling with heterozygotes is very sensitive to subtle differences in relative growth rates and, by extrapolation, to affinity.

We used a collection of ~5900 yeast strains with a heterozygous deletion marked with two unique, 20 bp, oligonucleotide molecular bar codes. The relative abundance of each strain is measured by amplifying the bar

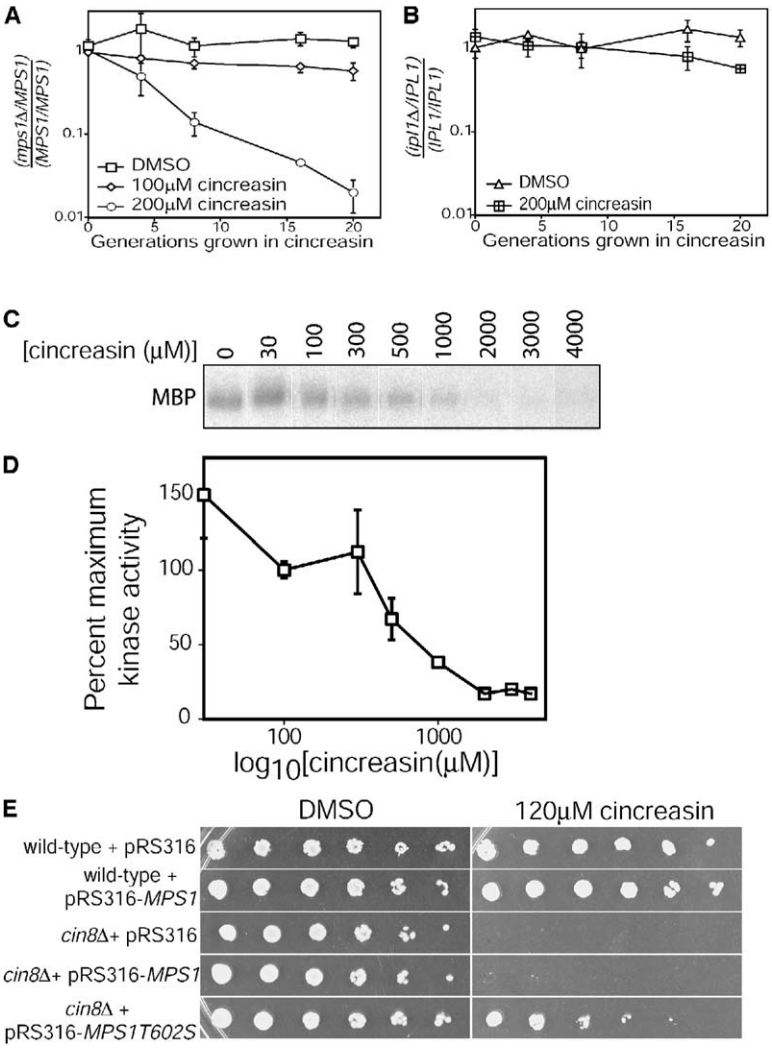

Figure 4. Mps1 Is a Target of Cincreasin

(A) *MPS1/mps1Δ* heterozygotes are sensitive to cincreasin when grown in competition with wild-type yeast in the absence of any other perturbation. After 20 generations, the *MPS1/mps1Δ* strain was 50-fold less abundant than at time zero.

(B) The *IPL1/ipl1Δ* heterozygous deletion strain is only slightly sensitive to 200 μM cincreasin when grown in competition with wild-type yeast. Viability assays were performed in triplicate, and the mean plus standard deviation of the ratio of the indicated strains is plotted.

(C and D) Mps1 is an in vitro target of cincreasin. Mps1 kinase (Mps1-GST) was assayed for its ability to phosphorylate myelin basic protein in vitro in the presence of the indicated concentrations of cincreasin. Kinase assays were performed in triplicate, and the mean plus standard deviation of the image density (determined with a phosphoimager) is plotted in (D). Similar results were obtained for the autophosphorylation activity of Mps1 (Figure S4).

(E) The *MPS1-T602S* kinase-domain mutation confers resistance to cincreasin. Fivefold serial dilutions of isogenic MATa wild-type or *cin8Δ* strains with the indicated plasmids were grown on plates containing either 0.1% DMSO alone or with 120 μM cincreasin for 2 days and photographed.

codes and hybridizing them to an oligonucleotide microarray [25–27]. We identified heterozygous deletions sensitive to cincreasin by treating the collection with the inhibitor and measuring the growth rates over 16 generations relative to those of a DMSO-treated control (see Supplemental Experimental Procedures for details). No statistically significant sensitive strains were identified at 100 μM cincreasin. At 200 μM and 400 μM, 53 and 106 sensitive strains were identified. All 53 mutants sensitive at 200 μM were also sensitive at 400 μM; 52 of these 53 strains were sensitive when individually retested on plates (data not shown). We found that the strain heterozygous for *MPS1* was among the most sensitive in the genome (Table S2; ranked second). Moreover, the *MPS1* heterozygote was the most sensitive of the known checkpoint strains (Figures S2A and S2B). Because cincreasin is similar to indolinones, a known class of kinase inhibitors, and *MPS1* has mitotic- and spindle-checkpoint functions, we hypothesized that *MPS1* could be a target of cincreasin.

We confirmed that *MPS1/mps1Δ* heterozygotes are sensitive to cincreasin by comparing the growth rate of *MPS1/mps1Δ* cells with wild-type yeast in a competitive-growth assay. Because another protein kinase, Ipl1, is also required for the tension-sensing branch of the spindle checkpoint, we included this strain as a control. Equal numbers of log-phase wild-type and mutant cells were mixed, and compound or DMSO was added to the medium. After 20 generations of growth in the presence of cincreasin, the *MPS1/mps1Δ* heterozygote was 50-fold less abundant in the culture (Figure 4A). In contrast, the *IPL1/ipl1Δ* heterozygote was only slightly sensitive to 200 μM cincreasin (Figure 4B). We also found that overexpression of *MPS1* on a 2 micron vector decreases the sensitivity of *cin8Δ* cells to cincreasin and partially restores the ability of cells with short linear chromosomes to arrest at the spindle checkpoint in the presence of cincreasin (data not shown). Taken together, these data suggest that Mps1 is an important target of cincreasin.

Mps1 as a Target of Cincreasin

To confirm that Mps1p is a target of cincreasin, we performed an in vitro kinase assay on purified Mps1p. Mps1p kinase activity is inhibited in vitro by cincreasin

with an IC_{50} of approximately 700 μM, with complete inhibition at 1 mM (Figures 4C and 4D; Figure S4). A separate test of Ipl1 failed to show inhibition of kinase activity in vitro (S. Biggins and S. Tatsutani, personal communication). Cincreasin completely inhibits growth of wild-type cells at a concentration of 1 mM (Figure S2), although given the compound's simple chemical structure, it is likely to inhibit several targets at this concentration. Lower concentrations of cincreasin (200 μM), which affect chromosome segregation and the response of the spindle checkpoint to chromosomes that are not under tension, do not affect viability or doubling time significantly (data not shown). The modest kinase inhibition seen in vitro is consistent with the idea that cincreasin only partially inhibits Mps1 in vivo. Mps1 plays an essential role in duplicating the spindle-pole body, the yeast equivalent of the centrosome, and because our screen identified nonlethal compounds that allow cells to proliferate because they inhibit the spindle checkpoint, these could not have been strong Mps1 inhibitors. Our results suggest that sensing of tension and attachment by the spindle checkpoint require different levels of Mps1 activity. This suggestion agrees with recent observations on quantitatively varying Mps1 activity: (1) more Mps1 activity is necessary for centrosome duplication than the spindle checkpoint [28]; (2) overexpression of Mps1 activates the spindle checkpoint [29]; and (3) *mps1-1* is partially defective for kinase activity at 23°C [30] and is synthetically lethal with mutations in *CIN8* [31] but is not defective in sensing attachment defects at 23°C [8]. Subtle defects in Mps1 activity could abrogate the tension response while preserving the response to severe attachment defects. Additional experiments with more-potent Mps1 inhibitors or a newly-described analog-sensitive allele of Mps1 [32] to carefully correlate the activity of Mps1 with the tension and attachment responses of the checkpoint should shed light on this issue.

To support our conclusion that Mps1 is a target of cincreasin, we identified dominant mutants, in the kinase domain of *MPS1*, that confer resistance to cincreasin. Six different alleles of *MPS1* render *cin8Δ* cells resistant to cincreasin (Table S3). Three of the six alleles contained an identical change in a single amino acid at position 602 (*T602S*), and we confirmed that this is the sole amino acid change required for the dominant suppression (Figure 4E). A wild-type *MPS1* on the same centromere (CEN) vector was incapable of suppression. *MPS1*-T602S mildly suppresses the cincreasin sensitivity of *bub1Δ* cells (data not shown), and this mutation does not inhibit *MPS1* function because the mutant protein suppresses the temperature sensitivity of an *mps1-1* mutant (data not shown). Threonine 602 is a residue whose homolog in cAMP-dependent protein kinase plays an important role in positioning a catalytic aspartic acid, making it likely that mutating this residue could affect the strength of cincreasin binding or increase the catalytic activity of Mps1 [33]. Taken together, our genetic and biochemical data suggest that Mps1 is an important target of cincreasin and that the lethality caused by cincreasin in *cin8Δ* mutants is due to a direct effect on the Mps1 protein.

Our findings are consistent with recent findings with an ATP-analog-sensitive allele of Mps1 and explain the wide range of deletion mutants that are sensitive to cincreasin. Cells completely lacking Mps1 kinase activity have severe defects in mitotic-spindle formation, sister-kinetochore positioning at metaphase, and chromosome segregation during anaphase, in addition to defects in the spindle checkpoint [32]. How Mps1 might specifically affect the tension-sensing machinery, including potential interactions with Ipl1 [14] and Sgo1 [10] in the checkpoint response, is not yet known.

Mps1 Kinase Inhibitors in Mammalian Cells

We tested the effect of cincreasin on mammalian cells. We were unable to inhibit the spindle-checkpoint response to microtubule depolymerization by nocodazole or inhibition of the kinesin Eg5 by monastrol in mammalian cells (R.K.D., unpublished data). Nevertheless, the conserved roles of Mps1 in mitosis and meiosis [34] suggest that a more potent and specific inhibitor of Mps1 would be a useful tool to probe cell division in mammalian cells. Many tumors display chromosomal instability, in which they gain or lose chromosomes and become aneuploid. For most tumors, the molecular basis of this instability is unknown. Some tumors are defective for the spindle checkpoint and carry mutations in *hBUB1*, whereas many others have a functional spindle checkpoint [35]. We suggest that both spindle-checkpoint-defective tumors and tumors defective for other aspects of chromosome segregation may be highly sensitive to inhibition of Mps1. Moreover, Mps1 kinase inhibitors may add a synergistic toxic effect to current chemotherapeutics that target the mitotic spindle or to newer drugs in development, including Aurora kinase inhibitors [36] and Eg5 kinesin inhibitors [37], that target other proteins required for mitosis.

Supplemental Data

Supplemental Data including Supplemental Experimental Procedures, four Supplemental Figures, and four Supplemental Tables are available with this article online at http://www.current-biology.com/cgi/content/full/15/11/■■■/DC1/.

Acknowledgments

We thank Christina Cuomo for discussion and help with the TAG3 microarrays and statistical analysis, Marion Shonn Dorer for comments on the manuscript and experimental advice, Bodo Stern, Alex Szidon, Scott Schulyer, Vahan Indjeian, and members of the Murray lab for experimental advice and discussion, and Mark Winey for reagents and helpful discussion. We thank Sue Biggins and Sean Tatsutani for sharing their unpublished results. This work was supported by funding from the National Institutes of Health grants GM043987 (A.W.M.), GM62566 (Institute of Chemistry and Cell Biology [ICCB]/A.W.M./T.J.M.), and CA96470 (W.H.W./A.W.M.). R.K.D. was supported by a Howard Hughes Medical Institute postdoctoral grant for physician-scientists.

Received: February 3, 2005
Revised: April 28, 2005
Accepted: May 3, 2005
Published: June 7, 2005

References

1. Li, R., and Murray, A.W. (1991). Feedback control of mitosis in budding yeast. Cell *66*, 519–531.

2. Hoyt, M.A., Trotis, L., and Roberts, B.T. (1991). S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. Cell *66*, 507–517.
3. Musacchio, A., and Hardwick, K.G. (2002). The spindle checkpoint: Structural insights into dynamic signalling. Nat. Rev. Mol. Cell Biol. *3*, 731–741.
4. Rieder, C.L., Cole, R.W., Khodjakov, A., and Sluder, G. (1995). The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. J. Cell Biol. *130*, 941–948.
5. Li, X., and Nicklas, R.B. (1995). Mitotic forces control a cell cycle checkpoint. Nature *373*, 630–632.
6. Nicklas, R.B., Ward, S.C., and Gorbsky, G.J. (1995). Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. J. Cell Biol. *130*, 929–939.
7. Stern, B.M., and Murray, A.W. (2001). Lack of tension at kinetochores activates the spindle checkpoint in budding yeast. Curr. Biol. *11*, 1462–1467.
8. Weiss, E., and Winey, M. (1996). The S. cerevisiae SPB duplication gene *MPS1* is part of a mitotic checkpoint. J. Cell Biol. *132*, 111–123.
9. Wells, W.A.E., and Murray, A.W. (1996). Aberrantly segregating centromeres activate the spindle assembly checkpoint in budding yeast. J. Cell Biol. *133*, 75–84.
10. Indjeian, V.B., Stern, B.M., and Murray, A.W. (2005). The centromeric protein Sgo1 is required to sense lack of tension on mitotic chromosomes. Science *307*, 130–133.
11. Pereira, G., Hofken, T., Grindlay, J., Manson, C., and Schiebel, E. (2000). The Bub2p spindle checkpoint links nuclear migration with mitotic exit. Mol. Cell *6*, 1–10.
12. Fraschini, R., Formenti, E., Lucchini, G., and Piatti, S. (1999). Budding yeast Bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from Mad2. J. Cell Biol. *145*, 979–991.
13. Fesquet, D., Fitzpatrick, P.J., Johnson, A.L., Kramer, K.M., Toyn, J.H., and Johnston, L.H. (1999). A Bub2p-dependent spindle checkpoint pathway regulates the Dbf2p kinase in budding yeast. EMBO J. *18*, 2424–2434.
14. Biggins, S., and Murray, A.W. (2001). The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes Dev. *15*, 3118–3129.
15. Spencer, F., Gerring, S.L., Connelly, C., and Hieter, P. (1990). Mitotic chromosome transmission mutatns in Saccharomyces cervisiae. Genetics *124*, 237–249.
16. Heiter, P., Pridmore, D., Hegemann, J.H., Thomas, M., Davis, R.W., and Philippsen, P. (1985). Functional selection and analysis of yeast centromeric DNA. Cell *42*, 913–921.
17. Cheeseman, I.M., Drubin, D.G., and Barnes, G. (2002). Simple centromere, complex kinetochore: Linking spindle microtubules and centromeric DNA in budding yeast. J. Cell Biol. *157*, 199–203.
18. Parsons, A.B., Brost, R.L., Ding, H., Li, Z., Zhang, C., Sheikh, B., Brown, G.W., Kane, P.M., Hughes, T.R., and Boone, C. (2004). Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways. Nat. Biotechnol. *22*, 62–69.
19. Jones, M.H., Bachant, J.B., Castillo, A.R., Giddings, T.H., Jr., and Winey, M. (1999). Yeast Dam1p is required to maintain spindle integrity during mitosis and interacts with the Mps1p kinase. Mol. Biol. Cell *10*, 2377–2391.
20. Jones, M.H., He, X., Giddings, T.H., and Winey, M. (2001). Yeast Dam1p has a role at the kinetochore in assembly of the mitotic spindle. Proc. Natl. Acad. Sci. USA *98*, 13675–13680.
21. Enquist-Newman, M., Cheeseman, I.M., Van Goor, D., Drubin, D.G., Meluh, P.B., and Barnes, G. (2001). Dad1p, third component of the Duo1p/Dam1p complex involved in kinetochore function and mitotic spindle integrity. Mol. Biol. Cell *12*, 2601–2613.
22. Hardwick, K.G., Li, R., Mistrot, C., Chen, R.H., Dann, P., Rudner, A., and Murray, A.W. (1999). Lesions in many different spindle components activate the spindle checkpoint in the budding yeast Saccharomyces cerevisiae. Genetics *152*, 509–518.
23. Straight, A.F., Belmont, A.S., Robinett, C.C., and Murray, A.W. (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Curr. Biol. *6*, 1599–1608.
24. King, R.W. (1999). Chemistry or biology: Which comes first after the genome is sequenced? Chem. Biol. *6*, R327–R333.
25. Lum, P.Y., Armour, C.D., Stepaniants, S.B., Cavet, G., Wolf, M.K., Butler, J.S., Hinshaw, J.C., Garnier, P., Prestwich, G.D., Leonardson, A., et al. (2004). Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. Cell *116*, 121–137.
26. Giaever, G., Flaherty, P., Kumm, J., Proctor, M., Nislow, C., Jaramillo, D.F., Chu, A.M., Jordan, M.I., Arkin, A.P., and Davis, R.W. (2004). Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. Proc. Natl. Acad. Sci. USA *101*, 793–798.
27. Giaever, G., Shoemaker, D.D., Jones, T.W., Liang, H., Winzeler, E.A., Astromoff, A., and Davis, R.W. (1999). Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat. Genet. *21*, 278–283.
28. Fisk, H.A., Mattison, C.P., and Winey, M. (2003). Human Mps1 protein kinase is required for centrosome duplication and normal mitotic progression. Proc. Natl. Acad. Sci. USA *100*, 14875–14880.
29. Hardwick, K.G., Weiss, E., Luca, F.C., Winey, M., and Murray, A.W. (1996). Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. Science *273*, 953–956.
30. Lauze, E., Stoelcker, B., Luca, F.C., Weiss, E., Schutz, A.R., and Winey, M. (1995). Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. EMBO J. *14*, 1655–1663.
31. Geiser, J.R., Schott, E.J., Kingsbury, T.J., Cole, N.B., Totis, L.J., Bhattacharyya, G., He, L., and Hoyt, M.A. (1997). Saccharomyces cerevisiae genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways. Mol. Biol. Cell *8*, 1035–1050.
32. Jones, M.H., Huneycutt, B.J., Pearson, C.G., Zhang, C., Morgan, G., Shokat, K., Bloom, K., and Winey, M. (2005). Chemical Genetics Reveals a Role for Mps1 Kinase in Kinetochore Attachment during Mitosis. Curr. Biol. *15*, 160–165.
33. Yang, J., Ten Eyck, L.F., Xuong, N.H., and Taylor, S.S. (2004). Crystal structure of a cAMP-dependent protein kinase mutant at 1.26A: new insights into the catalytic mechanism. J. Mol. Biol. *336*, 473–487.
34. Winey, M., and Huneycutt, B.J. (2002). Centrosomes and checkpoints: The MPS1 family of kinases. Oncogene *21*, 6161–6169.
35. Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1998). Genetic instabilities in human cancers. Nature *396*, 643–649.
36. Harrington, E.A., Bebbington, D., Moore, J., Rasmussen, R.K., Ajose-Adeogun, A.O., Nakayama, T., Graham, J.A., Demur, C., Hercend, T., Diu-Hercend, A., et al. (2004). VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. Nat. Med. *10*, 262–267.
37. Mayer, T.U., Kapoor, T.M., Haggarty, S.J., King, R.W., Schreiber, S.L., and Mitchison, T.J. (1999). Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. Science *286*, 971–974.