

# Interactions Between the Kinetochore Complex and the Protein Kinase A Pathway in *Saccharomyces cerevisiae*

Lina Ma,<sup>\*,†</sup> Krystina Ho,<sup>\*,‡</sup> Nina Piggott,<sup>\*</sup> Zongli Luo,<sup>\*</sup> and Vivien Measday<sup>\*,1</sup>

<sup>\*</sup>Wine Research Centre, Faculty of Land and Food Systems, <sup>†</sup>Genetics Graduate Program, and <sup>‡</sup>Graduate Program in Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

**ABSTRACT** The kinetochore is a large structure composed of multiple protein subcomplexes that connect chromosomes to spindle microtubules to enable accurate chromosome segregation. Significant advances have been made in the identification of kinetochore proteins and elucidation of kinetochore structure; however, comparatively little is known about how cellular signals integrate with kinetochore function. In the budding yeast *Saccharomyces cerevisiae*, the cyclic AMP protein kinase A signaling pathway promotes cellular growth in response to glucose. In this study, we find that decreasing protein kinase A activity, either by overexpressing negative regulators of the pathway or deleting the upstream effector *Ras2*, improves the viability of *ipl1* and *spc24* kinetochore mutants. *Ipl1*/Aurora B is a highly conserved kinase that corrects attachment of sister kinetochores that have attached to the same spindle pole, whereas *Spc24* is a component of the conserved *Ndc80* kinetochore complex that attaches directly to microtubules. Unexpectedly, we find that kinetochore mutants have increased phosphorylation levels of protein kinase A substrates, suggesting that the cyclic AMP protein kinase A signaling pathway is stimulated. The increase in protein kinase A activity in kinetochore mutants is not induced by activation of the spindle checkpoint or a metaphase delay because protein kinase A activity remains constant during an unperturbed cell cycle. Finally, we show that lowering protein kinase A activity can rescue the chromosome loss defect of the inner kinetochore *ndc10* mutant. Overall, our data suggest that the increased protein kinase A activity in kinetochore mutants is detrimental to cellular growth and chromosome transmission fidelity.

## KEYWORDS

budding yeast  
protein kinase A  
kinetochore  
chromosome  
segregation  
spindle  
checkpoint

Although the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) is able to utilize various carbon sources, glucose is the preferred carbon source. Upon addition of glucose to the cell, the cyclic AMP protein kinase A (cAMP-PKA) pathway is activated, and adenylate cyclase, called *Cyr1* in yeast, catalyzes the synthesis of cAMP from ATP. In *S. cerevisiae*, the activity of adenylate cyclase is stimulated by the small G proteins *Ras1* and *Ras2* and a G protein coupled receptor system

[supporting information, Figure S1, (Thevelein and de Winder 1999)]. *Cdc25* and *Sdc25* are two Ras guanine nucleotide exchange factor proteins that switch GDP-bound Ras to GTP-bound Ras, whereas *Ira1* and *Ira2* are redundant GTPase activating proteins that inactivate Ras by hydrolysis of the bound GTP to GDP (Dechant and Peter 2008; Tamaki 2007). The cAMP produced by adenylate cyclase binds *Bcy1*, which is an inhibitory subunit of PKA. In budding yeast, PKA is a heterotetramer of two catalytic subunits (any two of *Tpk1*, 2 or 3) and two *Bcy1* subunits. Once cAMP binds *Bcy1*, the two catalytic subunits are released from *Bcy1*, which allows these subunits to phosphorylate multiple target proteins, including phosphorylation and inhibition of the stress response transcription factors *Msn2* and *Msn4* (Gelade *et al.* 2003). cAMP accumulation in yeast is downregulated by both a low (*Pde1*) and high (*Pde2*) affinity phosphodiesterase, which hydrolyze cAMP to AMP (Nikawa *et al.* 1987; Sass *et al.* 1986).

In addition to mediating the cellular response to glucose, the PKA pathway also has cell cycle regulatory roles. For example, PKA activity mediates mitotic arrest in response to DNA damage by regulating

Copyright © 2012 Ma *et al.*

doi: 10.1534/g3.112.002675

Manuscript received March 16, 2012; accepted for publication May 16, 2012

This is an open-access article distributed under the terms of the Creative Commons Attribution Unported License (<http://creativecommons.org/licenses/by/3.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Supporting information is available online at <http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.112.002675/-/DC1>

<sup>1</sup>Corresponding author: Wine Research Centre, Room 325, 2205 East Mall, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

E-mail: vmeasday@mail.ubc.ca

phosphorylation of Cdc20 (Searle *et al.* 2004). Cdc20 is a specificity factor of the anaphase promoting complex (APC), and multiple studies suggest that PKA is an inhibitor of the APC (Anghileri *et al.* 1999; Bolte *et al.* 2003; Heo *et al.* 1999; Irniger *et al.* 2000). The PKA pathway has also been implicated in chromosome segregation. Three studies have demonstrated a potential interaction between the kinetochore, a large structure composed of multiple protein complexes that connects spindle microtubules to chromosomes, and the cAMP-PKA pathway. The inner kinetochore is comprised of the CBF3 centromere-binding complex and a modified nucleosome (Choy *et al.* 2012; Westermann *et al.* 2007). Sgt1, which is required for assembly of the CBF3 inner kinetochore complex, physically interacts with Cyr1 and upregulates the activity of the cAMP-PKA pathway (Dubacq *et al.* 2002). Overexpression of negative regulators of the cAMP-PKA pathway rescues the lethality of kinetochore mutants (Li *et al.* 2005; Magtanong *et al.* 2011). However, no systematic analysis has been performed to analyze the effect of increasing or decreasing PKA activity on multiple kinetochore mutants, including spindle checkpoint active and inactive alleles, or what impact a kinetochore defect might have on PKA activity.

In this study, we find that decreasing PKA activity rescues the viability of strains carrying mutations in the highly conserved Ipl1/Aurora B kinase and the Ndc80 kinetochore complex. We also show that reduction of PKA activity rescues the chromosome loss defect of the inner kinetochore *ndc10-1* mutant. Unexpectedly, we find that *ipl1*, *spc24*, and *ndc10-1* kinetochore mutants have a high level of PKA activity that is not due to spindle checkpoint activation or metaphase arrest. We propose that the high level of PKA activity in kinetochore mutants is partially responsible for the chromosome loss and growth defects in these strains.

## MATERIALS AND METHODS

### Yeast strains, plasmids, and media

The yeast strains used in this study are described in Table S1. The *pRS326-PDE2* plasmid was obtained from a 2  $\mu$  yeast genomic DNA library (Connelly and Hieter 1996) as a high copy suppressor of *spc24-9* lethality on 0.1M HU plates at 33° (Ma *et al.* 2007). The genomic DNA coordinates for the *PDE2* insert are Chr XV 1011626–1019437. A subclone was constructed that contained only the *PDE2* ORF (Chr XV 1013176–1015806), which also rescued *spc24-9* lethality (data not shown). The *BCY1* plasmid (*p416-GPD-BCY1*) and vector control (*p416-GPD*) were kind gifts from Kevin Morano (Trott *et al.* 2005). The *RAS2<sup>val19</sup>* plasmid was a kind gift from Paul Herman (Ramachandran and Herman 2011). The liquid media were rich medium (YPD) or supplemental minimal medium (SC) (Kaiser *et al.* 1994). The plates for the carbon source spot assays were glucose (2%), glycerol (2%), acetate (1%), ethanol (3%), and YEP (no added carbon source except for the yeast extract and peptone used in YPD media).

### Western blots

For Figure 3 and Figure 4, wild-type, *spc24-10*, *spc24-9*, *spc24-8*, *spc24-8 mad2Δ*, *mad2Δ*, *ndc10-1*, and *ipl1-321* cells were grown to mid-logarithmic phase at 25° (Figure 3A) or the semipermissive temperature of 31.5° (Figure 3B) in YPD medium. Wild-type cells harboring the *RAS2<sup>val19</sup>* plasmid were grown in SC-URA medium. 15 mL of culture was harvested, and the pellet was washed once with cold dH<sub>2</sub>O and lysed immediately. Forty micrograms of protein lysate was loaded on a 8% SDS-PAGE gel. For Figure 5A, wild-type cells were grown in YPD to log phase at 25°, synchronized in G1 with mating pheromone ( $\alpha$ -factor, 5  $\mu$ g/mL) for 2 hr, and released at 25°. Time

points were taken at 0 min, 30 min, and every 15 min thereafter for 120 min. The pellet from each 15 mL of culture was washed once with cold dH<sub>2</sub>O, frozen at –80°, and lysed the next day. For Figure 5B, wild-type cells were grown to mid-logarithmic phase in YPD medium, then treated with 20  $\mu$ g/mL nocodazole or without nocodazole (Log) for 2 hr at 25° before harvesting. *Msn2* phosphorylation was detected with  $\alpha$ -P-CREB (S133) antibody (1:1000, Cell Signaling), *Msn2* protein was detected with an  $\alpha$ -*Msn2* antibody (1:10,000, kind gift from Francisco Estruch), *Pgk1* protein levels were detected with an  $\alpha$ -*Pgk1* antibody (1:10,000, Invitrogen), phosphorylation of PKA substrates was detected with a phospho-PKA substrate antibody ( $\alpha$ -sub,1:5,000, Cell Signaling #9624), and *Clb2* was detected with an  $\alpha$ -*Clb2* antibody (1:5,000, Santa Cruz). For Figure 3, the blot was cut into two parts. The bottom part was probed with  $\alpha$ -*Pgk1*; the top part was first probed with  $\alpha$ -P-CREB, stripped for 30 min at 60° with Tris-SDS buffer [62.5 mM Tris HCl (pH 6.8), 2% SDS, 100 mM  $\beta$ -Mercaptoethanol], and then probed with  $\alpha$ -*Msn2*. For Figure 4, the blot was probed with  $\alpha$ -sub and then probed with  $\alpha$ -*Pgk1* after stripping. Images were captured with a ChemiDoc MP imaging system (Bio-Rad), and quantification was performed using Image Lab software (Bio-Rad). At least three independent experiments were performed for each Western blot. For Figures 3 and 4, a one-way ANOVA test was used to determine which mutants had significantly different phosphorylation levels from wild-type. For Figure 5, an ANOVA with Tukey's post-hoc analysis was done to compare values from all time points, except for time point zero (which has lower PKA activity due to pheromone treatment), with each other.

### DAPI staining and budding index

For each time point in Figure 5A, 1 mL of cells was spun down at 2000 rpm, fixed with 70% ethanol for 1 hr at room temperature, resuspended in PBS, and stored at 4°. For microscopy imaging, cells were lightly sonicated and mixed well with DAPI (final concentration 50 ng/mL). Cells were categorized into four groups: (1) G1 cells with no bud; (2) G2/S cells with a small bud; (3) metaphase cells with the nucleus at the bud neck; and (4) anaphase/telophase cells with a divided nucleus. Two hundred cells were counted for each time point.

### Chromosome fragment loss assay

*ndc10-1* and *ndc10-1 ras2Δ* mutants carrying a chromosome fragment (CF) (CFIII TRP) were grown over night in SC-TRP media to select for the CF, and then ~12,000 colonies were spread onto SC plates with limiting adenine as described (Koshland and Hieter 1987). *ndc10-1 pRS416 CFIII TRP* and *ndc10-1 pRS416-BCY1 CFIII TRP* strains were grown overnight in SC-TRP-URA media to select for the plasmid and CF, and then ~6,000 colonies were spread onto SC-URA plates (to select for the plasmid) with limiting adenine. Plates were incubated at 25° for 3 days and then incubated for 2 days at 4° to develop the red pigment. Colonies that were all red (CF lost while plating), half red/half white (CF lost during first cell division), and total colonies were counted.

## RESULTS

### Reduction of PKA activity alters the viability of kinetochore mutants:

The Ndc80 complex is a highly conserved essential kinetochore complex composed of Ndc80, Nuf2, Spc24, and Spc25 in which the Ndc80/Nuf2 subcomplex binds directly to microtubules and the Spc24/Spc25 subcomplex interacts with the kinetochore (Tooley and Stukenberg 2011). We previously performed a genome-wide synthetic

lethal (SL) screen with three phenotypically distinct alleles of the *SPC24* gene and identified SL or synthetic sick interactions between multiple *spc24* alleles and two negative regulators of the cAMP-PKA pathway, *ira2Δ* and *pde2Δ* (Montpetit *et al.* 2005). Consistent with our previous SL data, we found that overexpression of *PDE2*, which encodes the high affinity cAMP phosphodiesterase that converts cAMP to AMP (Sass *et al.* 1986), rescued the temperature-sensitive (Ts) lethality of *spc24-8*, *spc24-9*, and *spc24-10* mutants (Figure 1, A and B). Likewise, overexpression of *BCY1*, which negatively regulates PKA, also rescued the Ts lethality of all three *spc24* mutants (Figure 1, A and B) (Toda *et al.* 1987). The genetic interactions between negative regulators of the PKA pathway and *spc24* mutants suggest that high PKA activity is particularly detrimental to strains with defective kinetochore-microtubule attachments.

To determine whether other kinetochore mutants were affected by PKA activity, we overexpressed *BCY1* and *PDE2* in the *ipl1-321* mutant, which suffers from inappropriate attachment of sister kinetochores to the same spindle pole (Biggins and Murray 2001; Cheeseman *et al.* 2002; Tanaka *et al.* 2002). We found that the growth of the *ipl1-321* mutant was modestly rescued by *BCY1* or *PDE2* overexpression (Figure 1C). However, overexpression of *BCY1* or *PDE2* was detrimental to a *ndc10-1* mutant that lacks an assembled kinetochore at a restrictive temperature [Figure 1C, (Goh and Kilmartin 1993)].

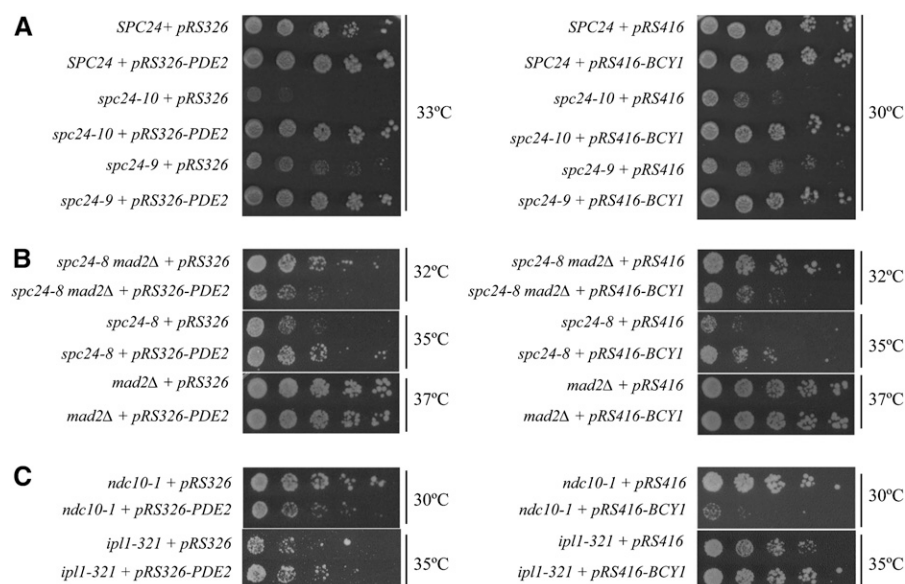
The small GTP-binding protein Ras2 interacts with adenylate cyclase and stimulates production of cAMP (Field *et al.* 1990; Suzuki *et al.* 1990). Consistent with the *BCY1* and *PDE2* overexpression data, we found that the double *spc24 ras2Δ* and *ipl1-321 ras2Δ* mutants grew better than *spc24* or *ipl1-321* single mutants, whereas the *ndc10-1 ras2Δ* double mutant grew more poorly (Figure 2, A and C). Therefore, lowering PKA activity is beneficial to mutants that have kinetochore-microtubule attachment defects but is detrimental to strains that have no kinetochore assembled. To determine how kinetochore mutants respond to an increase in PKA activity, we introduced a dominant allele of Ras2, *RAS2<sup>val19</sup>*, which activates the PKA pathway, into *ipl1-321*, *ndc10-1*, and *spc24* mutant strains. As expected, the temperature sensitivity of *ipl1-321* and *spc24* strains was exacerbated by the dominant *RAS2<sup>val19</sup>* allele and, surprisingly, so was the Ts of the *ndc10-1* strain (Figure 2D). Therefore increasing

PKA activity is detrimental to mutants with either no kinetochore or kinetochore-microtubule attachment defects.

The kinetochore monitors its state of microtubule attachment and signals to the spindle checkpoint to prevent anaphase onset in the presence of incorrectly attached or unattached kinetochores. Depending on the nature of the mutation, a kinetochore mutant may or may not activate the spindle checkpoint and arrest the cell cycle in metaphase. For example, of our three *spc24* alleles, only *spc24-8* cells are able to activate the spindle checkpoint at a restrictive temperature (Montpetit *et al.* 2005). To determine whether decreasing PKA activity can bypass the requirement for the spindle checkpoint, we impaired the spindle checkpoint in *spc24-8* by deletion of the *Mad2* checkpoint protein. The *spc24-8 mad2Δ* strain was SL at 34°, but the SL was rescued by deletion of *RAS2*, suggesting that lowering PKA levels may allow for partial bypass of the spindle checkpoint (Figure 2B). However, overexpression of *PDE2* and *BCY1* did not rescue the viability of the *spc24-8 mad2Δ* double mutant but instead exacerbated the growth defect (Figure 1B). In addition, increasing PKA activity by expressing *RAS2<sup>val19</sup>* decreased the restrictive temperature of *spc24-8 mad2Δ* (Figure 2D). These data, combined with the fact that decreasing PKA activity rescues both checkpoint active (*spc24-8*) and checkpoint defective (*spc24-9*, *spc24-10*) kinetochore mutants, suggest that lowering PKA activity does not improve the growth of kinetochore mutants due to restoration of the spindle checkpoint.

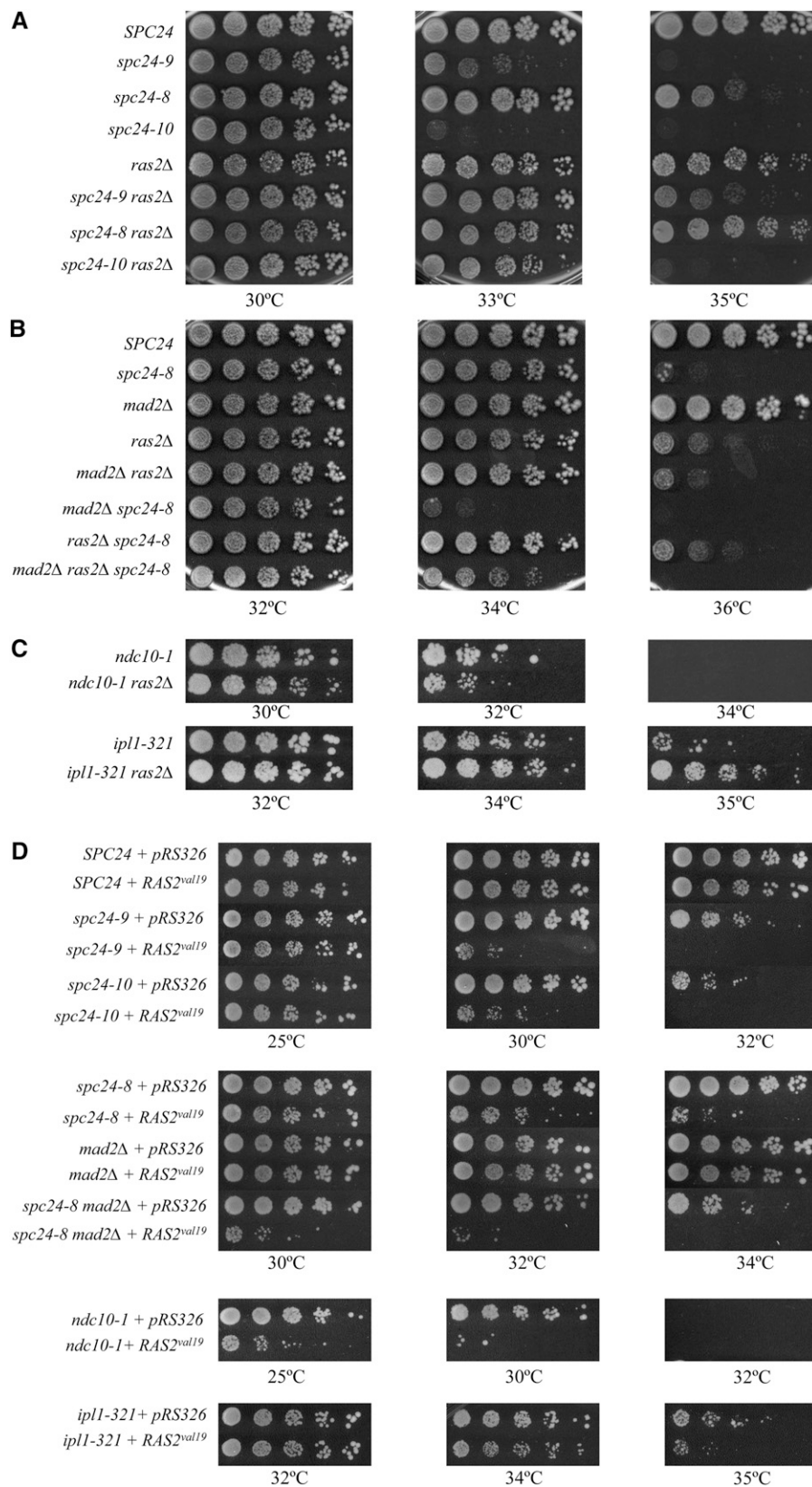
### Chromosome loss defects in *ndc10-1* mutants are suppressed by deletion of *RAS2*

*spc24*, *ndc10-1*, and *ipl1-321* mutants have defects in chromosome stability (Biggins *et al.* 2001; Goh and Kilmartin 1993; Montpetit *et al.* 2005). The chromosome fragment (CF) loss assay is a colony color-based sector assay to determine whether a strain is able to maintain a nonessential CF in the cell (Koshland and Hieter 1987). Colonies that contain the CF are white, whereas colonies that lose the CF are red. If a haploid cell loses the CF in the first cell division, the colony is half-red and half-white (red/white half-sector). We asked whether reduction of PKA activity was able to rescue the chromosome segregation defects of kinetochore mutants using the CF loss assay. We overexpressed *BCY1* or deleted *RAS2* in *spc24-9*, *ndc10-1*, and *ipl1-321* strains carrying a CF and screened qualitatively for rescue



**Figure 1** Inhibition of the cAMP-PKA pathway is beneficial to *ipl1-321* and *spc24* kinetochore mutants but detrimental to the *ndc10-1* kinetochore mutant. (A–C) Cell dilution assay of wild-type cells and indicated mutants carrying the plasmid vectors *pRS416-GPD* (*pRS416*), *pRS416-GPD-BCY1* (*pRS416-BCY1*), *pRS326*, or *pRS326-PDE2* grown on SC-URA plates at the indicated temperatures for 2–4 days.





**Figure 2** Expression of the *RAS2<sup>Val19</sup>* dominant allele is detrimental to all kinetochore mutants, whereas deletion of *RAS2* rescues a subset of kinetochore mutants. (A–C) Cell dilution assay of the indicated strains grown on YPD plates at the indicated temperatures for 3 days. (D) Cell dilution assay of wild-type cells and the indicated mutants carrying the plasmid vectors *pRS326* or *pRAS2<sup>val19</sup>* grown on SC-URA plates at the indicated temperatures for 3 days.

of CF loss at a variety of temperatures compared with kinetochore mutant alone. We determined that *ndc10-1* CF loss, at a permissive temperature, was rescued by both overexpression of *BCY1* or deletion

of *RAS2*, whereas *ipl1-321* and *spc24-9* CF loss was rescued by neither at a permissive or semirestrictive temperature. We next performed quantitative analysis of CF loss in *ndc10-1* strains by growing cells

in media to select for the CF, then plating on nonselective minimal media that contains limiting adenine, which enriches for the red color that arises when cells lose the CF. We scored for colonies that lost the CF in the first cell division (half-white, half-red) and for complete CF loss (red colonies, Table 1). The CF loss events in *ndc10-1* were dramatically suppressed by deletion of *RAS2* (~20-fold) with a  $1.3 \times 10^{-2}$  CF loss rate in *ndc10-1* compared with a  $6.4 \times 10^{-4}$  CF loss rate in *ndc10-1 ras2Δ* mutants (Table 1). Overexpression of *BCY1* also suppressed CF loss rates in *ndc10-1* cells by ~4-fold (Table 1,  $8.3 \times 10^{-3}$  CF loss events in *ndc10-1* cells compared with  $1.9 \times 10^{-3}$  CF loss events in *ndc10-1* cells carrying *BCY1*). In addition to reduction of half sector colonies, inhibition of PKA activity reduced the frequency of red colonies (total CF loss) in *ndc10-1* cells by ~17-fold in the absence of *RAS2* and ~2-fold when *BCY1* was overexpressed (Table 1). In summary, we have found that decreasing PKA activity rescues chromosome loss in *ndc10-1* mutants at a permissive temperature.

### Kinetochores mutants have increased levels of PKA activity

One rationale for the rescue of kinetochore defects when PKA activity is reduced is that PKA activity is elevated in kinetochore mutants. We employed two markers to determine if PKA levels are perturbed in *spc24* mutants – the PKA dependent phosphorylation of the Msn2 transcription factor, and the phosphorylation profile of PKA substrates. We analyzed Msn2 phosphorylation in wild-type vs. kinetochore mutants using a phospho-CREB antibody that specifically recognizes PKA-dependent phosphorylation (Gorner *et al.* 2002). As a control, we analyzed Msn2 phosphorylation in wild-type strain expressing the dominant *RAS2<sup>val19</sup>* allele. We quantified Msn2 PKA-specific phosphorylation compared with total Msn2 protein levels for all the samples. At a permissive temperature (25°) all *spc24* mutants had similar Msn2 PKA-phosphorylation levels to wild-type cells (Figure 3A), whereas at a semipermissive temperature (31.5°), *spc24-9* and *spc24-8 mad2Δ* cells had significantly higher (2.5- to 3-fold) Msn2 PKA-dependent phosphorylation levels compared with wild-type cells (Figure 3B). As expected, the phosphorylation of Msn2 in cells carrying *RAS2<sup>val19</sup>* was dramatically increased by 5.7-fold at 25° and 4.1-fold at 31.5° compared with wild-type cells due to hyperactivation of the PKA pathway. Notably, *ipl1-321* mutants had a 1.6-fold increase in Msn2 PKA-phosphorylation compared with the wild-type cells at 25°, and *ndc10-1* had a 3.7-fold increase at 31.5°. Therefore, cells with defective kinetochore assembly or kinetochore-microtubule attachments have higher PKA activity. There was a reduction in total Msn2 protein levels in *spc24-10*, *ndc10-1*, and *ipl1-321* cells, possibly due to a reduction in overall protein synthesis in these mutants at a semirestrictive temperature or because defects at the kinetochore affect Msn2 stability. Because Pgc1 levels are similar in all kinetochore mutants tested and *spc24-10* mutants are not sensitive to cycloheximide (data not shown), we do not think that protein synthesis is reduced in *spc24-10*, *ndc10-1*, and *ipl1-321* mutants.

Next, we examined the phosphorylation profile of PKA substrates in the kinetochore mutants using a phospho-PKA substrate antibody (α-sub). Strains were grown to log phase at permissive (25°C) and semipermissive (31.5°) temperatures, lysates were generated, and Western blot analysis was performed. A wild-type strain expressing *RAS2<sup>val19</sup>* was used as control to detect PKA substrates. We used a chemiluminescence detection system and exposed the blot for a very short time (15 sec) before any protein band was saturated. Our image software detected six prominent PKA-phosphorylated substrates at 25° and five substrates at 31.5° that migrated between 70 kD and 200 kD (Figure 4, A and B). All six bands have a stronger signal in *Ras2<sup>val19</sup>* compared with the wild-type cells at 25°, suggesting that these substrates are PKA phosphorylated. We quantified two proteins, Pa and Pb, as representative PKA substrates for all the strains. At 25°, all mutants have Pa phosphorylation levels similar to wild-type cells. However, when compared with wild-type cells, the phosphorylation level of Pb is increased by 2.6-fold in *spc24-8 mad2Δ*, 3.8-fold in *ndc10-1* cells, and 7.2-fold in *Ras2<sup>val19</sup>* cells. At 31.5°, all the mutants, except for *spc24-8* and *mad2Δ* mutants, have significantly increased phosphorylation of Pa by ~2-fold compared with wild-type cells, whereas the phosphorylation of Pb does not change. We noticed that the 100 kD PKA substrate was absent from the *ndc10-1* strain and the ~160 kD band had reduced mobility (Figure 4B, *ndc10-1* lane). Because Ndc10 is a 112 kD protein, we wondered whether Ndc10 was the 100 kD PKA substrate that was absent in *ndc10-1* strains. However, we analyzed the PKA profile of strains carrying tagged versions of Ndc10 (Ndc10-13Myc and Ndc10-3HA), which should decrease mobility of the 100 kD band, and we did not detect any change in migration, suggesting that Ndc10 is not the 100 kD PKA substrate (data not shown). Therefore the 100 kD substrate could be an Ndc10-interacting protein that is destabilized at a semirestrictive temperature in the *ndc10-1* strain. Overall, these data demonstrate that PKA substrates have increased levels of phosphorylation in multiple kinetochore mutant strains, suggesting that PKA activity is elevated in these strains.

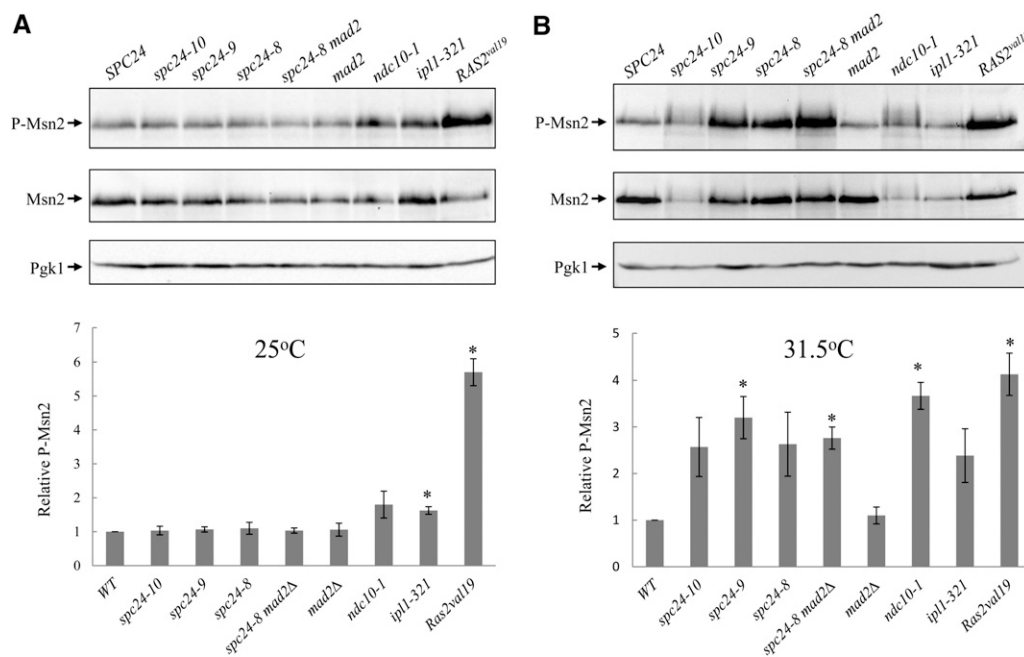
### PKA activity does not fluctuate during an unperturbed cell cycle

Strains defective in kinetochore function tend to accumulate in either metaphase or anaphase with a 2N population of DNA. Therefore, it is possible that we detect an increase in PKA activity in kinetochore mutants due to an overall increase in PKA activity in metaphase or anaphase. To address this possibility, we investigated Msn2 phosphorylation levels during the cell cycle. Wild-type cells were arrested in G1 phase at 25° with the mating pheromone α-factor, released into the cell cycle for 30 min and time points taken every 15 min until the cells had divided. We monitored Msn2 PKA-dependent phosphorylation, Msn2 protein levels, protein levels of the Clb2 mitotic cyclin, and nuclear division. At 0 min, Msn2 phosphorylation levels were low because mating pheromone inhibits adenylate cyclase (Liao and

■ Table 1 Reduction of PKA activity rescues CF loss in the *ndc10-1* kinetochore mutant

Genotype (MATa)	Total Colonies	Chromosome Loss (Red)	Chromosome Loss in First Division (Red/White Half Sectors)
<i>ndc10ΔHIS3 ndc10-1::kanMX6 CFIII</i>	11,632	$6.9 \times 10^{-2}$ (801) <sup>a</sup>	$1.3 \times 10^{-2}$ (143)
<i>ndc10ΔHIS3 ndc10-1::kanMX6 ras2ΔLEU2 CFIII</i>	11,118	$4.1 \times 10^{-3}$ (46)	$6.3 \times 10^{-4}$ (7)
<i>ndc10ΔHIS3 ndc10-1::kanMX6 CFIII pRS416</i>	5,926	$1.8 \times 10^{-2}$ (104)	$8.3 \times 10^{-3}$ (49)
<i>ndc10ΔHIS3 ndc10-1::kanMX6 CFIII pRS416-BCY1</i>	6,409	$9.7 \times 10^{-3}$ (62)	$1.9 \times 10^{-3}$ (12)

<sup>a</sup> Numbers in brackets represent the total number of red or red/white colonies.



**Figure 3** Kinetochore mutants have higher levels of Msn2 PKA-dependent phosphorylation. Cells were grown to mid-logarithmic phase at 25°C (A) or the semipermissive temperature of 31.5°C (B) and lysed for immunoblot analysis. The blots were probed with  $\alpha$ -P-CREB antibody to detect PKA-dependent Msn2 phosphorylation (P-Msn2) and  $\alpha$ -Msn2 and  $\alpha$ -Pgk1 as loading controls. One representative blot is shown out of three independent experiments. The bar graphs are the relative ratio of P-Msn2/total Msn2 analyzed from the three independent experiments. The value of 1 was assigned to the ratio of P-Msn2/total Msn2 in the wild-type cells. The asterisk represents values that are significantly different from the wild-type value ( $P < 0.05$ ).

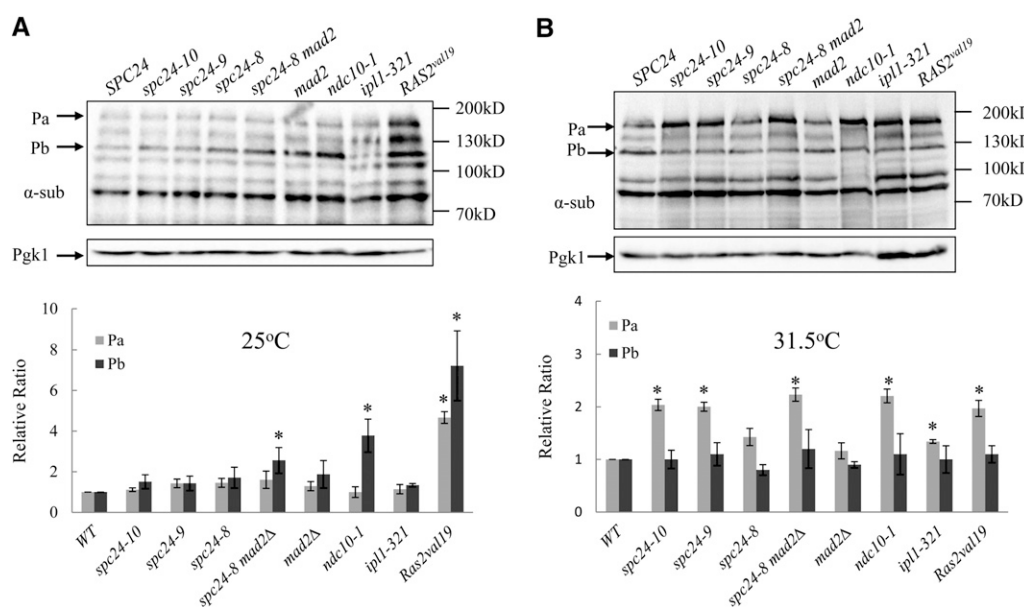
Thorner 1980). The metaphase-to-anaphase transition occurred between 75 and 90 min as Clb2 levels peaked and the nuclei divided (Figure 5A). Although Msn2 PKA-dependent phosphorylation appeared to increase at 75 to 90 min, so did Msn2 protein levels; therefore, when quantitated, no increase in Msn2 phosphorylation was detected at the metaphase-to-anaphase transition (Figure 5A). In fact, no significant difference was detected in Msn2 PKA-dependent phosphorylation between 30 and 120 min, suggesting that PKA activity remains constant during the cell cycle.

To specifically analyze Msn2 PKA-dependent phosphorylation in a population of metaphase-arrested cells, we treated wild-type cells with the microtubule depolymerizing agent nocodazole. Interestingly, the Msn2 PKA-dependent phosphorylation was significantly reduced upon nocodazole treatment (Figure 5B). In summary, our cell-cycle

analysis suggests that Msn2 PKA-dependent phosphorylation does not specifically increase at the metaphase-to-anaphase transition. On the contrary, under conditions of cell-cycle arrest in G1 phase (mating pheromone treatment) or metaphase arrest (nocodazole treatment), Msn2 PKA-dependent phosphorylation is decreased. Therefore, our data suggest that the increase in PKA activity in kinetochore mutants is not simply due to an increase in PKA activity at the metaphase-to-anaphase transition.

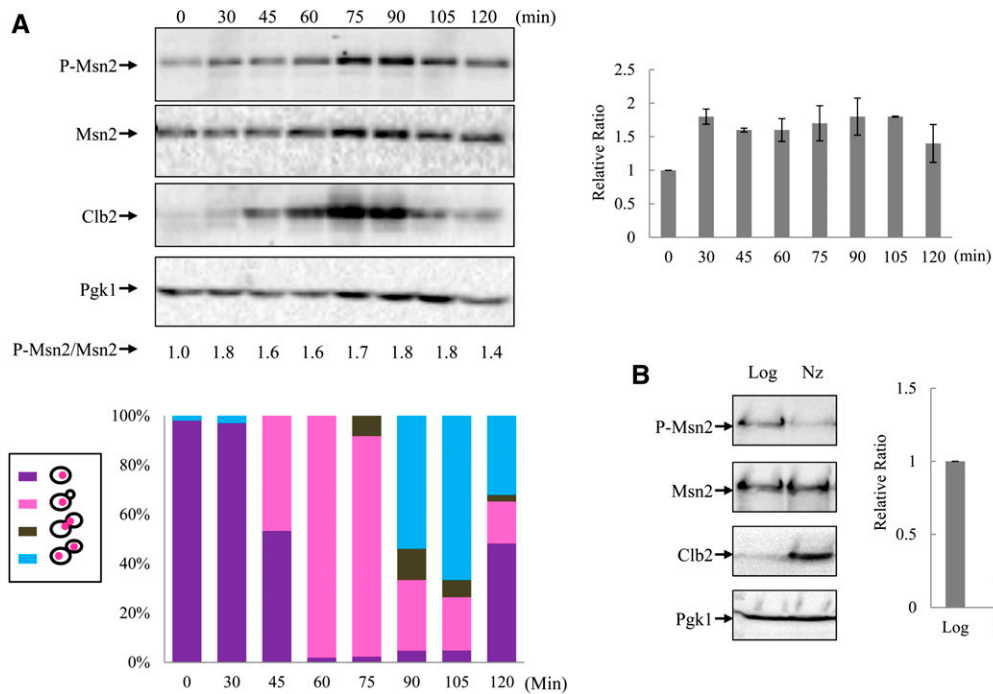
### Nonfermentable carbon sources permit growth at a higher restrictive temperature for mutants of the Ndc80 complex

Cells grown in glucose media are presumed to have high PKA activity, whereas cells growing in nonfermentable carbon sources are presumed



**Figure 4** PKA substrates have higher levels of phosphorylation in kinetochore mutants. (A) The lysates described in Figure 3 were probed with the anti-PKA substrate antibody ( $\alpha$ -sub) and  $\alpha$ -Pgk1 as a loading control. Two protein bands from the  $\alpha$ -sub blot, Pa and Pb, were selected for quantification. One representative blot from three independent experiments is shown. Bar graphs are the relative ratio of Pa/Pgk1 or Pb/Pgk1 averaged from three independent experiments. The value of 1 was assigned to the ratio of Pa/Pgk1 or Pb/Pgk1 in the wild-type cells. The asterisk represents values that are significantly different from the wild-type value ( $P < 0.05$ ).





**Figure 5** Msn2 PKA-dependent phosphorylation is not cell cycle regulated. (A) Wild-type cells were synchronized in G1 with mating pheromone ( $\alpha$ -factor, 5  $\mu$ g/mL) for 2 hr and released at 25°C. Samples were taken at the indicated time points and analyzed for Msn2 PKA-dependent phosphorylation by immunoblotting (top panel) and for nuclear division by microscopy (bottom panel). The Western blot was probed with P-Msn2 ( $\alpha$ -CREB),  $\alpha$ -Msn2,  $\alpha$ -Clb2, or  $\alpha$ -Pgk1. The bar graph (top right) is the relative ratio of P-Msn2/total Msn2 analyzed from two independent experiments. A value of 1 was assigned to the ratio of P-Msn2/total Msn2 in the wild-type cells. (B) Wild-type cells were grown to mid-logarithmic phase at 25°C in YPD medium and either kept in log phase (Log) or treated with 20  $\mu$ g/mL nocodazole (Nz) for 2

hr at 25°C. Western blots were probed with P-Msn2 ( $\alpha$ -CREB),  $\alpha$ -Msn2, and  $\alpha$ -Clb2 as a marker for cell-cycle arrest. The asterisk represents the significant difference between the P-Msn2 levels upon Nz treatment vs. log phase cells ( $P < 0.05$ ).

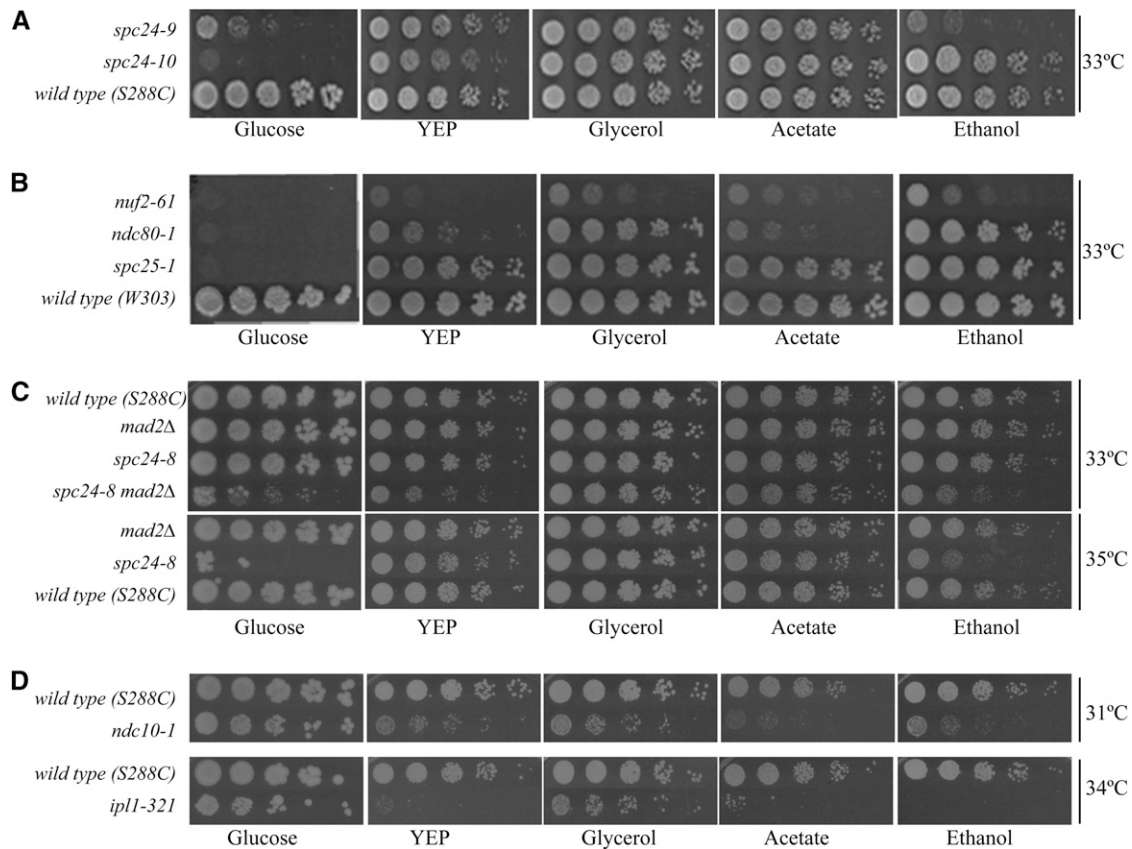
to have low PKA activity (Thevelein and de Winder 1999). Because reducing PKA activity increases the restrictive temperature of *spc24* mutants, we tested the growth of *spc24* mutants by plate assay on glucose vs. nonfermentable carbon sources (glycerol, acetate, and ethanol) or no added carbon source except for the yeast extract and peptone in the media (YEP) (Figure 6). *spc24* mutants grew at a higher restrictive temperature on almost every media tested compared with glucose media (Figure 6, A and C). Notably, *spc24-8* is rescued on nonfermentable carbon sources when the spindle checkpoint is active (*spc24-8*) or inactive (*spc24-8 mad2 $\Delta$* , Figure 6C). Therefore, exposing *spc24* mutants to glucose, which triggers high PKA activity, is inhibitory to growth. We asked whether the glucose growth inhibition phenotype was specific to *spc24* mutants or shared with other mutants in the Ndc80 complex. Ts mutants of other proteins of the Ndc80 complex, *spc25-1*, *ndc80-1*, and *nuf2-61*, were also rescued by growth on nonfermentable carbon sources (Figure 6B). To determine whether the glucose sensitivity was specific to the Ndc80 complex, we spotted *ndc10-1* and *ipl1-321* cells onto nonfermentable carbon sources (Figure 6D). Contrary to mutants in the Ndc80 complex, the fitness of the *ndc10-1* mutant was optimal on glucose media, which is consistent with decreased fitness of *ndc10-1* cells when PKA activity is reduced (Figures 1C, 2C, and 6D). Surprisingly, glucose was also the preferred carbon source of the *ipl1-321* mutant, despite fact that lowering PKA activity improves the fitness of *ipl1-321* cells (Figures 1C, 2C, and 6D). A previous report also found that the *ipl1-2* mutant cannot be rescued by plating on media that slows growth, such as nonfermentable carbon sources (Tatchell *et al.* 2011). Because the growth rate of yeast is slower on nonfermentable carbon sources, it was possible that the rescue of *spc24* mutants on nonglucose plates was due to a reduction in growth rate. Therefore, we tested growth of a subset of *spc24* mutants on limiting nitrogen, cycloheximide, and rapamycin plates, all of which cause a reduction in growth rate. We did not detect any

rescue of the *spc24* Ts phenotype in any condition (data not shown). Therefore, *spc24* mutants are rescued specifically by growth on non-glucose carbon sources and not generally by slowing cell growth rate.

## DISCUSSION

This work stemmed from the initial observation that decreasing cAMP-PKA activity, either by overproducing *BCY1* or *PDE2* or by deleting *RAS2*, rescued the lethality of *spc24* kinetochore mutants (Figures 1 and 2). In addition, we had previously identified genetic interactions between *pde2* and *ira2*, which are mutants of negative regulators of the cAMP-PKA pathway, and *spc24* mutants (Montpetit *et al.* 2005). A simple model of these observations is that the cAMP-PKA pathway inhibits kinetochore function. However, our data suggest that the interaction between the cAMP-PKA pathway and the kinetochore is more complex. Inhibition of PKA activity is beneficial to the growth of *spc24* and *ipl1-321* mutants but detrimental to the *ndc10-1* mutant, in which no kinetochore is assembled, and the *spc24-8 mad2 $\Delta$*  double mutant, in which the spindle checkpoint has been abolished (Figure 1). In addition, all kinetochore mutants, except for the spindle checkpoint proficient *spc24-8* mutant, have higher levels of PKA activity (Figures 3 and 4). The increased PKA activity is not due to accumulation of cells in metaphase or anaphase because during an unperturbed cell cycle, PKA-dependent Msn2 phosphorylation levels remain constant (Figure 5A). As well, metaphase-arrested cells have low levels of PKA activity (Figure 5B). Therefore, defects in the kinetochore, and not cell cycle arrest, cause an increase in PKA activity. Finally, we show that reduction of PKA activity rescues chromosome loss defects of the *ndc10-1* mutant at a permissive temperature (Table 1). Therefore, the kinetochore is highly sensitive to fluctuations in PKA activity.

We demonstrate that, in addition to suppression of lethality by lowering PKA activity, mutants of the Ndc80 kinetochore complex are sensitive to glucose, and their growth defects are suppressed on nonfermentable carbon sources (Figure 6). It was shown previously



**Figure 6** Strains carrying mutations in the Ndc80 kinetochore complex are rescued by growth on nonglucose carbon sources. Cell dilution assays were performed on the following media: 2% glucose, no added carbon source (YEP), 2% glycerol, 1% acetate, and 3% ethanol. (A) *spc24-9*, *spc24-10* and isogenic wild-type strain (S288C background); (B) Ndc80 complex mutants *nuf2-61*, *ndc80-1*, *spc25-1* and isogenic wild-type strain (W303 background); (C) *spc24-8*, *mad2Δ*, *spc24-8 mad2Δ* and isogenic wild-type strain (S288C); and (D) *ndc10-1*, *ipl1-321* and isogenic wild-type strain (S288C). Plates were incubated at the indicated temperatures for 3 days. Different temperatures are shown depending on the restrictive temperature of each Ts allele.

that mutants of the APC are also suppressed by reducing Ras signaling and growth on nonglucose carbon sources (Irniger *et al.* 2000). Therefore, we expected that all kinetochore mutants that were rescued by reducing PKA signaling would preferentially grow on nonglucose carbon sources. However, we tested a variety of kinetochore mutants in addition to the mutants presented here and found no strict correlation between growth rescue by inhibition of PKA signaling and growth rescue on nonglucose carbon sources. For example, a strain carrying a mutation in *CTF13*, which codes for a protein in the CBF3 inner kinetochore complex, displayed no growth changes upon overexpression of *BCY1* but was rescued by growth on glycerol or galactose media (data not shown). The growth of the *ipl1-321* mutant is improved upon deletion of *RAS2* or overexpression of *BCY1* but not when plated on nonfermentable carbon sources (Figures 1, 2, and 6). Although the viability of all mutants of the Ndc80 kinetochore complex was rescued by reducing PKA signaling or plating on a nonglucose carbon source, mutants of other kinetochore complexes, such as the CBF3, COMA, and Dam1 complexes, displayed variable phenotypes (data not shown). Therefore, the sensitivity of kinetochore mutants to fluctuations in PKA activity is not strictly correlated to the presence of glucose in the media.

The chromosome loss defect of *ndc10-1* cells is reduced by 20-fold upon deletion of *RAS2* and 4-fold overexpression of *BCY1* at 25° (Table 1). However, when grown at 30°, we find that overexpression of *BCY1* causes lethality to *ndc10-1* strains and that *ndc10-1 ras2Δ*

strains grow more poorly than do *ndc10-1* strains at 32° (Figures 1C and 2C). At a permissive temperature, kinetochore complexes still associate with centromere DNA in the *ndc10-1* mutant, whereas at a restrictive temperature, no kinetochore complexes are able to assemble on the centromere (He *et al.* 2001; Janke *et al.* 2001; Nekrasov *et al.* 2003; Ortiz *et al.* 1999). Therefore, lowering PKA activity only rescues CF loss of the *ndc10-1* mutant when there is an assembled kinetochore that is able to attach to microtubules. Perhaps this is why we did not detect rescue of CF loss in *ipl1-321* or *spc24-9* mutants in which kinetochore-microtubule attachment is impaired.

We find that the *ipl1-321* growth defect is suppressed by overexpression of *PDE2* and *BCY1* or by deletion of *RAS2*, all of which lower PKA activity (Figures 1C and 2C). In addition, expression of the dominant *RAS<sup>val19</sup>* allele is detrimental to *ipl1-321* growth (Figure 2D). The rescue of *ipl1-321* growth defect by lowering PKA activity is intriguing in light of recent data demonstrating that reduction of target of rapamycin (TOR) complex 1 (TORC1) activity also suppresses the growth defect of an *ipl1-2* mutant (Tatchell *et al.* 2011). TOR and PKA are the two major signaling pathways that activate cell growth in response to nutrients by regulating processing, such as translation, ribosome biogenesis, and glucose metabolism (Smets *et al.* 2010; Soulard *et al.* 2009). TORC1 and PKA have been shown to regulate common target proteins, and recent data demonstrate that TOR can activate PKA toward a subset of substrates (Soulard *et al.* 2010). Reduction of TORC1 activity suppressed the chromosome loss

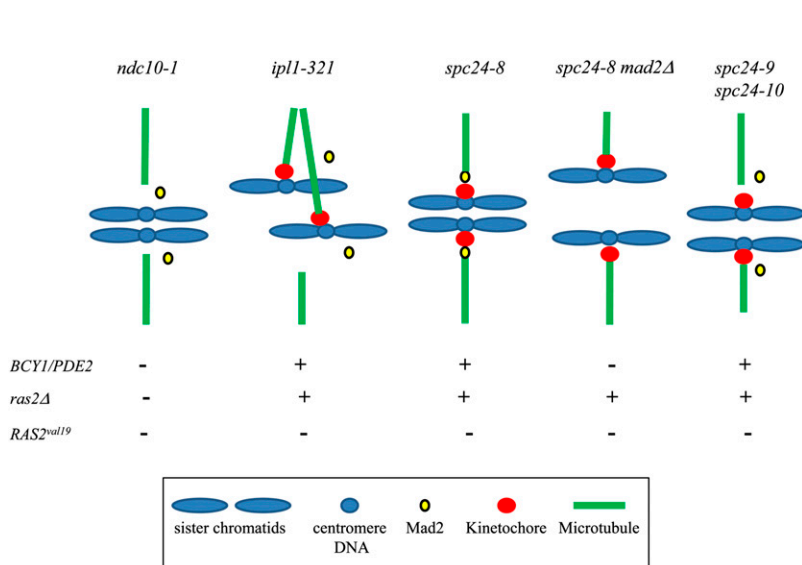


defect in *ipl1-2* mutants, whereas we did not detect rescue of CF loss in *ipl1-321* mutants upon reduction of PKA activity (Tatchell *et al.* 2011). Nonetheless, our data combined with Tatchell *et al.* (2011) strongly support a link between nutritional status and kinetochore function.

Activation of the PKA pathway is known to be inhibitory to the APC, possibly via phosphorylation of Cdc20 (Anghileri *et al.* 1999; Bolte *et al.* 2003; Irniger *et al.* 2000; Searle *et al.* 2004). For example, the growth defect of *apc10-22* mutants, which stabilizes Pds1, is suppressed by deletion of *RAS2* (Irniger *et al.* 2000). Stabilization of Pds1 (securin) prevents chromosome separation due to inhibition of Esp1 (separase). The APC is also inhibited, and Pds1 is stabilized upon activation of the spindle checkpoint by the interaction of the Mad2 checkpoint protein with Cdc20 (Clarke and Bachant 2008). However, no study has addressed the interaction between the PKA pathway and the spindle checkpoint. In this work, we monitored Msn2 PKA-dependent phosphorylation in cells arrested in metaphase due to induction of the spindle checkpoint upon nocodazole treatment (Figure 5B). We found that Msn2 PKA-dependent phosphorylation is reduced under these conditions, suggesting that PKA activity may be reduced during the spindle checkpoint. Interestingly, the only kinetochore mutant that did not display high levels of PKA activity was *spc24-8*, which arrests in metaphase due to activation of the spindle checkpoint (Montpetit *et al.* 2005) (Figures 3 and 4). It is possible that reducing PKA levels suppresses *spc24* and *ipl1* mutants due to spindle checkpoint activation; however, this does not explain why the viability of the *spc24-8* mutant, which is spindle checkpoint proficient, is improved by reducing PKA activity (Figures 1 and 2). Activation of the PKA pathway causes phosphorylation of the Msn2 nuclear localization sequence on serine residues, prevention of Msn2 nuclear import, and restoration of Msn2 to the cytoplasm (Gorner *et al.* 1998, 2002; Jacquet *et al.* 2003). Inhibition of PKA activity upon nocodazole treatment might evoke a general stress response that results in dephosphorylation of Msn2 and subsequent localization to the nucleus. In fact, microarray studies performed after treatment of yeast cells with benomyl, another microtubule poison, demonstrated that expression of Msn2 genes is induced (Lucau-Danila *et al.* 2005). Whether inhibition of PKA activity rescues *spc24* mutants due to activation of a Msn2-dependent stress response remains to be tested.

Because it is difficult to separate the spindle checkpoint response from a general cell stress response upon addition of microtubule poisons, deletion of the Mad2 spindle checkpoint protein is an alternative method to assess the spindle checkpoint. We find that overexpression of *BCY1* and *PDE2* only rescues viability of strains that have a partially assembled kinetochore and Mad2 present in the cell (Figure 7). For example, overexpression of *BCY1* and *PDE2* rescues the growth defect of the *spc24-8* (spindle checkpoint active) mutant but is detrimental to the growth of the *spc24-8 mad2Δ* (spindle checkpoint defective) mutant (Figures 1B and 7). The APC specificity factor Cdc20 is phosphorylated on PKA consensus sites after DNA damage, and inactivation of PKA accelerates Pds1 destruction (Searle *et al.* 2004). If phosphorylation of Cdc20 by PKA prevents an interaction between Cdc20 and Mad2, then decreasing PKA activity may enrich the Cdc20-Mad2 interaction and spindle checkpoint response. If Mad2 is not present, then decreasing PKA activity may accelerate Pds1 destruction, which would force *spc24-8 mad2* cells into anaphase with defective chromosome attachments. One complication to this argument is that deletion of *RAS2* rescues the growth defect of both *spc24-8* and *spc24-8 mad2Δ* strains (Figures 2B and 7). Likely, there is more than one mechanism by which a reduction in PKA activity rescues viability of kinetochore mutants.

An unexpected finding from our study is that kinetochore mutants have increased levels of PKA activity when grown at a semipermissive temperature (Figures 3 and 4). These data suggest that defects in the kinetochore may trigger activation of the PKA pathway, possibly via components of the cAMP-PKA pathway, that are nuclear localized. Bcy1, the PKA regulatory subunit, is predominantly in the nucleus when cells are grown in glucose (Griffioen *et al.* 2000). It remains to be determined whether a nuclear pool of Bcy1 or a nuclear-localized PKA catalytic subunit, such as Tpk1, interacts with the kinetochore and whether disruption of kinetochore function releases active Tpk1. It has been demonstrated that loss of one copy of *BCY1* in a diploid strain increases the rate of chromosome loss, suggesting that Bcy1 may be important for kinetochore function (Magtanong *et al.* 2011). How the kinetochore impinges upon the cAMP-PKA pathway and whether this function is conserved in higher eukaryotes will be an interesting subject of future research.



**Figure 7** Summary of kinetochore and PKA interactions. At a restrictive temperature, the *ndc10-1* mutant does not assemble a kinetochore; therefore, microtubules cannot attach and Mad2 cannot be recruited. The *ipl1-321* mutant has functional kinetochores that attach to the same pole, but Mad2 is not recruited (Gillett *et al.* 2004). The *spc24-8* mutant is checkpoint active; therefore, Mad2 is presumed to be properly localized to the kinetochore. The *spc24-8 mad2Δ* mutant lacks Mad2 and is spindle checkpoint defective; however, kinetochores are still present. The state of the kinetochore-microtubule interaction in *spc24-8 mad2Δ* cells has not been investigated. The kinetochores in *spc24-9* and *spc24-10* mutants have defects in microtubule attachment and are presumed to mislocalize Mad2 as the spindle elongates in both mutants, despite attachment defects. In addition, similar mutants in the Ndc80 complex mislocalize Mad2 (Gillett *et al.* 2004). Genetic interactions upon overexpression of *BCY1* or *PDE2*, deletion of *RAS2* (*ras2Δ*), or expression of *RAS2<sup>val19</sup>* in each kinetochore mutant is represented with a plus (+) sign if growth defects were improved and minus (-) sign if growth defects were exacerbated.

## ACKNOWLEDGMENTS

We would like to thank Merisa Mok for helping with colony counting for the CF loss assays. We would also like to thank Tony Bretscher for the gift of the *ras2Δ* strain, Francisco Estruch for the gift of the Msn2 antibody, Paul Herman for the gift of the *Ras2<sup>val19</sup>* plasmid, Brie Lavoie for the gift of the *ipl1-321* strain, Kevin Morano for the gift of the *BCY1* plasmid, and Kristin Baetz for critical comments on the manuscript. K. Ho was supported by a Canadian Institutes of Health Research (CIHR) Frederick Banting and Charles Best Canada Graduate Scholarship. This work was supported by CIHR operating grant MOP-84242 to V. Measday. V. Measday is a Canada Research Chair in enology and yeast genomics.

## LITERATURE CITED

- Anghileri, P., P. Branduardi, F. Sternieri, P. Monti, R. Visintin *et al.*, 1999 Chromosome separation and exit from mitosis in budding yeast: dependence on growth revealed by cAMP-mediated inhibition. *Exp. Cell Res.* 250: 510–523.
- Biggins, S., and A. W. Murray, 2001 The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. *Genes Dev.* 15: 3118–3129.
- Biggins, S., N. Bhalla, A. Chang, D. L. Smith, and A. W. Murray, 2001 Genes involved in sister chromatid separation and segregation in the budding yeast *Saccharomyces cerevisiae*. *Genetics* 159: 453–470.
- Bolte, M., P. Dieckhoff, C. Krause, G. H. Braus, and S. Irniger, 2003 Synergistic inhibition of APC/C by glucose and activated Ras proteins can be mediated by each of the Tpk1–3 proteins in *Saccharomyces cerevisiae*. *Microbiology* 149: 1205–1216.
- Cheeseman, I. M., S. Anderson, M. Jwa, E. M. Green, J. Kang *et al.*, 2002 Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. *Cell* 111: 163–172.
- Choy, J. S., P. K. Mishra, W. C. Au, and M. A. Basrai, 2012 Insights into assembly and regulation of centromeric chromatin in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* (in press).
- Clarke, D. J., and J. Bachant, 2008 Kinetochore structure and spindle assembly checkpoint signaling in the budding yeast, *Saccharomyces cerevisiae*. *Front. Biosci.* 13: 6787–6819.
- Connelly, C., and P. Hieter, 1996 Budding yeast SKP1 encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. *Cell* 86: 275–285.
- Dechant, R., and M. Peter, 2008 Nutrient signals driving cell growth. *Curr. Opin. Cell Biol.* 20: 678–687.
- Dubacq, C., R. Guerois, R. Courbeyrette, K. Kitagawa, and C. Mann, 2002 Sgt1p contributes to cyclic AMP pathway activity and physically interacts with the adenyl cyclase Cyr1p/Cdc35p in budding yeast. *Eukaryot. Cell* 1: 568–582.
- Field, J., H. P. Xu, T. Michaeli, R. Ballester, P. Sass *et al.*, 1990 Mutations of the adenyl cyclase gene that block RAS function in *Saccharomyces cerevisiae*. *Science* 247: 464–467.
- Gelade, R., S. Van de Velde, P. Van Dijk, and J. M. Thevelein, 2003 Multi-level response of the yeast genome to glucose. *Genome Biol.* 4: 233.
- Gillett, E. S., C. W. Espelin, and P. K. Sorger, 2004 Spindle checkpoint proteins and chromosome-microtubule attachment in budding yeast. *J. Cell Biol.* 164: 535–546.
- Goh, P. Y., and J. V. Kilmartin, 1993 NDC10: a gene involved in chromosome segregation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 121: 503–512.
- Gorner, W., E. Durchschlag, M. T. Martinez-Pastor, F. Estruch, G. Ammerer *et al.*, 1998 Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev.* 12: 586–597.
- Gorner, W., E. Durchschlag, J. Wolf, E. L. Brown, G. Ammerer *et al.*, 2002 Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. *EMBO J.* 21: 135–144.
- Griffioen, G., P. Anghileri, E. Imre, M. D. Baroni, and H. Ruis, 2000 Nutritional control of nucleocytoplasmic localization of cAMP-dependent protein kinase catalytic and regulatory subunits in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 275: 1449–1456.
- He, X., D. R. Rines, C. W. Espelin, and P. K. Sorger, 2001 Molecular analysis of kinetochore-microtubule attachment in budding yeast. *Cell* 106: 195–206.
- Heo, S. J., K. Tatebayashi, and H. Ikeda, 1999 The budding yeast cohesin gene SCC1/MCD1/RHC21 genetically interacts with PKA, CDK and APC. *Curr. Genet.* 36: 329–338.
- Irniger, S., M. Baumer, and G. H. Braus, 2000 Glucose and ras activity influence the ubiquitin ligases APC/C and SCF in *Saccharomyces cerevisiae*. *Genetics* 154: 1509–1521.
- Jacquet, M., G. Renault, S. Lallet, J. De Mey, and A. Goldbeter, 2003 Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in *Saccharomyces cerevisiae*. *J. Cell Biol.* 161: 497–505.
- Janke, C., J. Ortiz, J. Lechner, A. Shevchenko, A. Shevchenko *et al.*, 2001 The budding yeast proteins Spc24p and Spc25p interact with Ndc80p and Nuf2p at the kinetochore and are important for kinetochore clustering and checkpoint control. *EMBO J.* 20: 777–791.
- Kaiser, C., S. Michaelis, and A. Mitchell, 1994 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Koshland, D., and P. Hieter, 1987 Visual assay for chromosome ploidy. *Methods Enzymol.* 155: 351–372.
- Li, J. M., Y. Li, and S. J. Elledge, 2005 Genetic analysis of the kinetochore DASH complex reveals an antagonistic relationship with the ras/protein kinase A pathway and a novel subunit required for Ask1 association. *Mol. Cell Biol.* 25: 767–778.
- Liao, H., and J. Thorner, 1980 Yeast mating pheromone alpha factor inhibits adenylate cyclase. *Proc. Natl. Acad. Sci. USA* 77: 1898–1902.
- Lucau-Danila, A., G. Lelandais, Z. Kozovska, V. Tanty, T. Delaveau *et al.*, 2005 Early expression of yeast genes affected by chemical stress. *Mol. Cell Biol.* 25: 1860–1868.
- Ma, L., J. McQueen, L. Cuschieri, J. Vogel, and V. Measday, 2007 Spc24 and Stu2 promote spindle integrity when DNA replication is stalled. *Mol. Biol. Cell* 18: 2805–2816.
- Magtanong, L., C. H. Ho, S. L. Barker, W. Jiao, A. Baryshnikova *et al.*, 2011 Dosage suppression genetic interaction networks enhance functional wiring diagrams of the cell. *Nat. Biotechnol.* 29: 505–511.
- Montpetit, B., K. Thorne, I. Barrett, K. Andrews, R. Jadusingsh *et al.*, 2005 Genome-wide synthetic lethal screens identify an interaction between the nuclear envelope protein, Apq12p, and the kinetochore in *Saccharomyces cerevisiae*. *Genetics* 171: 489–501.
- Nekrasov, V. S., M. A. Smith, S. Peak-Chew, and J. V. Kilmartin, 2003 Interactions between centromere complexes in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 14: 4931–4946.
- Nikawa, J., P. Sass, and M. Wigler, 1987 Cloning and characterization of the low-affinity cyclic AMP phosphodiesterase gene of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 7: 3629–3636.
- Ortiz, J., O. Stemmann, S. Rank, and J. Lechner, 1999 A putative protein complex consisting of Ctf19, Mcm21, and Okp1 represents a missing link in the budding yeast kinetochore. *Genes Dev.* 13: 1140–1155.
- Ramachandran, V., and P. K. Herman, 2011 Antagonistic interactions between the cAMP-dependent protein kinase and Tor signaling pathways modulate cell growth in *Saccharomyces cerevisiae*. *Genetics* 187: 441–454.
- Sass, P., J. Field, J. Nikawa, T. Toda, and M. Wigler, 1986 Cloning and characterization of the high-affinity cAMP phosphodiesterase of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 83: 9303–9307.
- Searle, J. S., K. L. Schollaert, B. J. Wilkins, and Y. Sanchez, 2004 The DNA damage checkpoint and PKA pathways converge on APC substrates and Cdc20 to regulate mitotic progression. *Nat. Cell Biol.* 6: 138–145.
- Smets, B., R. Ghillebert, P. De Snijder, M. Binda, E. Swinnen *et al.*, 2010 Life in the midst of scarcity: adaptations to nutrient availability in *Saccharomyces cerevisiae*. *Curr. Genet.* 56: 1–32.
- Soulard, A., A. Cohen, and M. N. Hall, 2009 TOR signaling in invertebrates. *Curr. Opin. Cell Biol.* 21: 825–836.

- Soulard, A., A. Cremonesi, S. Moes, F. Schutz, P. Jenö *et al.*, 2010 The rapamycin-sensitive phosphoproteome reveals that TOR controls protein kinase A toward some but not all substrates. *Mol. Biol. Cell* 21: 3475–3486.
- Suzuki, N., H. R. Choe, Y. Nishida, Y. Yamawaki-Kataoka, S. Ohnishi *et al.*, 1990 Leucine-rich repeats and carboxyl terminus are required for interaction of yeast adenylate cyclase with RAS proteins. *Proc. Natl. Acad. Sci. USA* 87: 8711–8715.
- Tamaki, H., 2007 Glucose-stimulated cAMP-protein kinase A pathway in yeast *Saccharomyces cerevisiae*. *J. Biosci. Bioeng.* 104: 245–250.
- Tanaka, T. U., N. Rachidi, C. Janke, G. Pereira, M. Galova *et al.*, 2002 Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell* 108: 317–329.
- Tatchell, K., V. Makrantoni, M. J. Stark, and L. C. Robinson, 2011 Temperature-sensitive *ipl1-2*/Aurora B mutation is suppressed by mutations in TOR complex 1 via the Glc7/PP1 phosphatase. *Proc. Natl. Acad. Sci. USA* 108: 3994–3999.
- Thevelein, J. M., and J. H. de Winde, 1999 Novel sensing mechanisms and targets for the cAMP-protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Mol. Microbiol.* 33: 904–918.
- Toda, T., S. Cameron, P. Sass, M. Zoller, J. D. Scott *et al.*, 1987 Cloning and characterization of BCY1, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7: 1371–1377.
- Tooley, J., and P. T. Stukenberg, 2011 The Ndc80 complex: integrating the kinetochore's many movements. *Chromosome Res.* 19: 377–391.
- Trott, A., L. Shaner, and K. A. Morano, 2005 The molecular chaperone Sse1 and the growth control protein kinase Sch9 collaborate to regulate protein kinase A activity in *Saccharomyces cerevisiae*. *Genetics* 170: 1009–1021.
- Westermann, S., D. G. Drubin, and G. Barnes, 2007 Structures and functions of yeast kinetochore complexes. *Annu. Rev. Biochem.* 76: 563–591.

*Communicating editor: M. Tyers*