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The budding yeast silencing protein Sir1 is a functional component of centromeric chromatin

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Abstract

In fission yeast and multicellular organisms, centromere-proximal regions of chromosomes are heterochromatic, containing proteins that silence gene expression. In contrast, the relationship between heterochromatin proteins and kinetochore function in the budding yeast *Saccharomyces cerevisiae* remains largely unexplored. Here we report that the yeast heterochromatin protein Sir1 is a component of centromeric chromatin and contributes to mitotic chromosome stability. Sir1 recruitment to centromeres occurred through a novel mechanism independent of its interaction with the Origin Recognition Complex. Sir1 function at centromeres was distinct from its role in forming heterochromatin, because the Sir2-4 proteins were not associated with centromeric regions. Sir1 bound to Cac1, a subunit of Chromatin Assembly Factor-I, and helped to retain Cac1 at centromeric loci. These studies reveal that although budding yeast and mammalian cells employ fundamentally different mechanisms of forming heterochromatin, they both use silencing proteins to attract the histone deposition factor CAF-I to centromeric chromatin.

Introduction

Eukaryotic chromosome transmission during cell division depends largely on the formation of stable contacts between the mitotic spindle and chromosomes. Centromeres promote spindle attachment to chromosomes by nucleating assembly of kinetochores, nucleoprotein structures that bind microtubules. In the fission yeast *Schizosaccharomyces pombe* and in multicellular eukaryotes, heterochromatic domains flank all centromeres and are required for high-fidelity chromosome segregation (Karpen and Allshire 1997). Like other heterochromatic loci, these centromere-flanking domains are distinguished from euchromatin by their ability to silence the transcription of reporter genes in a position-dependent manner. Centromeric heterochromatin helps to position centromeres toward the opposing spindle poles, thereby fostering bipolar microtubule-kinetochore contacts (Bernard et al. 2001).

As in all eukaryotes, epigenetic gene silencing in the budding yeast *S. cerevisiae* is

mediated by heterochromatin (Loo and Rine 1995). However, the protein composition of the silencing machinery in the budding yeast *S. cerevisiae* stands in marked contrast to that of *S. pombe*. For example, budding yeast lack structural homologs of the Swi6 (HP1) and Clr4 (Su(var)3-9) proteins (Moazed 2001). Further, the methylation of Lys9 on histone H3, a signature of heterochromatin in *S. pombe* and other eukaryotic organisms, has not been detected in budding yeast (Briggs et al., 2001). Instead, transcriptional silencing of the cryptic mating-type loci *HMR* and *HML* in *S. cerevisiae* requires the Sir1-4 proteins (Loo and Rine 1995).

Two distinct nucleosome assembly factors, CAF-I and the Hir proteins, contribute to the specialized chromatin structures at both silent and centromeric loci in budding yeast (Kaufman et al. 1998; Sharp et al. 2002). Although position-dependent gene silencing has not been described at centromeres in budding yeast, the involvement of CAF-I and Hir proteins at centromeric chromatin led us to test whether Sir1-4 proteins were also present at centromeres. Surprisingly, we found that only the Sir1 protein (and not Sir2, 3, or 4) was associated with centromeres. Further, recruitment of Sir1 to centromeres and *HM* loci required distinct protein interactions. Sir1 interacted directly with the large subunit of CAF-I and was required together with Hir1 for normal levels of association of CAF-I with centromeres. Epistasis experiments determined that Sir1 prevented chromosome nondisjunction in a manner that was overlapping with Cac1 and Hir1. Therefore, although Sir1 was previously known to function only in the formation of heterochromatin at *HM* loci, these data demonstrate an unsuspected role for Sir1 in promoting chromosome stability during mitosis.

Results and Discussion

To explore the role of budding yeast silencing proteins in centromere function, chromatin immunoprecipitation (ChIP) was used to test whether the Sir1-4 proteins associated with centromeric regions. Sir1, but not Sir2, 3, or 4, was detected at both centromeric DNA and at *HM* loci (Figures 1A and 1B). Sir1 was enriched at all centromeres tested, which included *CEN1-CEN4*, *CEN11*, and *CEN16* (Figure 1A and data not shown). Control immunoprecipitations

confirmed that the enrichment of Sir1-HA at centromeres was dependent on both the presence of anti-HA antibody and the HA epitope on Sir1 (Figure 1A, lanes 2 and 3). Further, analysis of four negative control loci revealed that the association of *CEN* and *HM* loci with Sir1 was specific. For example, immunoprecipitation of Sir1-HA recovered only background levels of *ACT1*, subtelomeric regions on chromosome VI, and the transcriptionally active *MAT* locus. These data were consistent with previous genetic experiments that demonstrated that the cryptic mating loci *HMR* and *HML*, but not telomeres or the *MAT* locus are subject to transcriptional control by Sir1 (Loo and Rine 1995). Although Sir2-4 ChIP eluates contained significant amounts of the *HMR-E* silencer element and telomere-proximal DNA, levels of *CEN* DNA were quantitatively comparable to *MAT* and *ACT1* negative control loci (Figure 1A, 1B). All four Sir proteins were enriched ~13-17-fold at the *HMR-E* silencer element relative to *MAT*, but only Sir1 was enriched ~10-14-fold at six centromeres tested (Figure 1A, 1B).

The distribution of individual centromere-associated proteins varies across centromere-proximal chromatin. For example, cohesin subunits (Megee et al. 1999; Tanaka et al. 1999) and Cac1 (Sharp et al. 2002) are distributed across ~10 kb of centromeric chromatin; in contrast, the centromere-specific histone Cse4 and the CENP-C homolog Mif2 are tightly localized over only the 125 bp core centromeric DNA (Meluh et al. 1998). To determine whether the distribution of Sir1 was similar to either of these patterns, ChIP experiments were used to examine the 10 kb flanking CENIII. Like Cse4, Sir1 was tightly localized over core centromeric DNA (Figure 1C), suggesting that specific protein-protein interactions at the kinetochore resulted in recruitment of Sir1.

Cytological analysis was used as a second test of Sir1 centromere localization. Budding yeast centromeres are clustered together and generally appear as one or two foci depending on the stage of the cell cycle (Guacci et al. 1997). The Ndc10 protein, an essential component of kinetochores, displays a similar clustered localization pattern. In chromosome spread preparations, we observed that brightly staining Sir1 foci colocalized with Ndc10 (Figure 1D). Sir1 was also present in multiple weakly staining foci that did not colocalize with Ndc10, demonstrating that the

chromatin-associated pool of Sir1 included, but was not limited to, centromeric domains.

Because the function of the kinetochore is to ensure chromosome segregation during cell division, the centromeric localization of Sir1 suggested an undiscovered role for Sir1 in chromosome stability. To test this idea, we measured the rates of chromosome missegregation per cell division (Shero et al. 1991; Sharp et al. 2002) in wild type and *sir1*Δ cells. *sir1*Δ cells displayed an average 21-fold increase in the rate of chromosome loss compared to wild-type cells (wild-type: 2.0×10^{-5} +/- 0.44×10^{-5}; *sir1*Δ: 4.1×10^{-4} +/- 1.5×10^{-4}; n=3 experiments). In contrast, in *sir4*Δ cells the rate of chromosome loss was equivalent to that of wild type, consistent with earlier studies (*sir4*Δ: 2.2×10^{-5} +/- 2.2×10^{-6}; n=3 experiments; Palladino et al. 1993). These data demonstrate that defects in *HM* silencing *per se* do not cause chromosome missegregation, and that *sir1*Δ mutants display a chromosome loss phenotype not observed in another *sir* mutant. Further, these data are consistent with the localization of Sir1 but not the other Sir proteins to centromeric chromatin (Figures 1A-B), and support a role for Sir1 at centromeric chromatin that is distinct from its role in recruiting the Sir2-4 proteins to *HM* silencer elements.

Additional criteria were used to test whether *SIR1* interacted genetically with known kinetochore components. First, *SIR1* was found to interact with a gene essential for chromosome segregation. Specifically, deletion of *SIR1* increased the maximum permissive temperature of cells carrying a mutation in the centromere-specific histone Cse4 (Supplementary Figure 1, *cse4-107* allele; Chen et al. 2000), and caused greater resistance of *cse4-107* cells to the microtubule-depolymerizing drug benomyl. Second, genetic interactions between a nonessential kinetochore component and *SIR1* were also observed. Cells lacking the outer kinetochore protein Mcm19 are sensitive to benomyl, and this sensitivity was partially suppressed by deletion of *SIR1* (Ghosh et al. 2001; Supplementary Figure 1). Third, deletion of the spindle checkpoint gene *MAD2* in *sir1*Δ cells resulted in greatly increased chromosome loss rates. Consistent with earlier studies, *mad2*Δ cells caused an 10-fold increase in chromosome loss, but chromosome loss rates of *sir1*Δ *mad2*Δ cells were increased 270-fold relative to the wild type control (wild-type: 2.0×10^{-5} +/- 0.44×10^{-5}; *mad2*Δ: 2.1×10^{-4} +/- 3.5×10^{-5}; *sir1*Δ *mad2*Δ: 5.4×10^{-3} +/- 1.3×10^{-3}; n=3 experiments; Li and

Murray 1991; Warren et al. 2002). These data indicate that both *SIR1* and *MAD2* promote chromosome stability in unperturbed mitotic cell divisions. Together, these genetic interaction data demonstrate that Sir1 affects the fidelity of chromosome segregation.

Recruitment of Sir1 to *HM* silencers occurs through a direct interaction between Sir1 and the N-terminal region of the Orc1 subunit of ORC (Triolo and Sternglanz 1996; Fox et al. 1997; Zhang et al. 2002). Mutant forms of Sir1 (termed "srd", silencer recognition defective) that fail to interact with Orc1 can still recruit the other Sir proteins for silencing, but must be artificially tethered to an *HM* silencer to do so (Gardner et al. 1999; Gardner and Fox 2001). To test whether ORC also recruited Sir1 to centromeric regions, we performed ChIP experiments with cells containing HA-tagged wild-type Sir1 or two altered Sir1^{srd} proteins that contain single amino acid changes (Figure 2, lanes 1-3). We observed that Sir1^{srd} proteins did not associate efficiently with *HML* silencers, consistent with their previously characterized defects in ORC binding. In contrast, localization of Sir1^{srd} proteins was unperturbed at both *CEN16* and *CEN3* (Figure 2 and data not shown). Identical results were achieved when the localization of wild-type Sir1 was analyzed in a strain containing an N-terminally truncated Orc1 protein which is unable to bind Sir1 (Figure 2, lane 4; (Triolo and Sternglanz 1996; Gardner et al. 1999). Therefore, the localization of Sir1 to centromeres was independent of ORC, suggesting that a previously undetected set of interactions was required for this localization.

Next, we tested whether the centromeric association of Sir1 depended on intact kinetochore structure. Ndc10 acts at an early step in kinetochore assembly, because *ndc10* mutations abolish the centromere localization of every other kinetochore protein tested to date (Biggins and Walczak 2003). Therefore, Sir1 ChIP experiments were performed with strains containing either the temperature-sensitive *ndc10-1* mutation or the wild-type *NDC10* allele (Figure 2, lanes 6-9). At the restrictive temperature, centromere association of Sir1 was sharply decreased in *ndc10-1* cells, yet recruitment of Sir1 to the *HML* silencer was unchanged. In contrast, centromere association of Sir1 was independent of the outer kinetochore protein Mcm19 (data not shown). Therefore, the association of Sir1 with centromeric chromatin displayed distinct requirements for the integrity of

inner and outer kinetochore structure. Moreover, because Sir1 recruitment responded differently to *NDC10* and *ORC1* perturbations in a locus-specific manner, the targeting of Sir1 to heterochromatin and centromeric chromatin occurred via independent interactions.

In mammalian cells, a direct link between nucleosome assembly and heterochromatic gene silencing has been established: CAF-I is recruited to pericentric heterochromatin by direct interaction between the large subunit of CAF-I and HP1 (Murzina et al. 1999). The presence of both CAF-I (Sharp et al. 2002) and the silencing protein Sir1 (Figures 1 and 2) at budding yeast centromeres suggested that direct interactions might exist between CAF-I and Sir1 in yeast. Indeed, we detected a physical interaction between Sir1 and the large subunit of CAF-I (Cac1) in multiple assays. First, this interaction was observed *in vivo* using a yeast two-hybrid assay (Figure 3A). To determine whether Sir1 and Cac1 interact directly, the interaction was tested in a GST coprecipitation assay. *In vitro*-translated Sir1 co-precipitated with a GST-Cac1 fusion protein (Figure 3B) and required amino acids 87-306 on GST-Cac1. Additionally, Cac1 and Sir1 interacted when expressed in baculovirus-infected insect cells (Figure 3C). Coimmunoprecipitation of Sir1 with CAF-I occurred specifically in extracts prepared from cells expressing both Sir1 and CAF-I, and was antibody dependent. Together, these data demonstrated a direct interaction between Sir1 and the large subunit of CAF-I.

The chromatin-associated pool of CAF-I is enriched at centromeric regions (Sharp et al. 2002; Figure 4A). The physical interaction between Cac1 and Sir1 suggested that Sir1 might contribute to the centromere association of Cac1 or vice-versa. To test this, ChIP experiments were performed in cells lacking the *SIR1* gene. Although *sir1*Δ cells displayed wild-type levels of Cac1 centromere association, a marked reduction was observed in cells containing both *sir1*Δ and *hir1*Δ gene deletions (Figure 4A). In contrast, *cac1*Δ and *hir1*Δ mutations alone or in combination had no effect on the association of Sir1 with either *CEN16* or *HM* loci (data not shown). Western blot analysis of Cac1-HA immunoprecipitations showed similar expression levels and immunoprecipitation efficiency from all strains tested (Figure 4A, bottom panel), indicating that changes observed in the ChIP assay were not attributable to changes in cellular Cac1 protein levels.

Quantitation of Cac1-HA ChIP experiments demonstrated a ~3-fold reduction of Cac1 association with *CEN3* and *CEN16* in *sir1*Δ *hir1*Δ cells compared to the wild-type cells (Figure 4B). These data suggest that the stable association of Cac1 with centromeric loci is mediated by a complex network of protein interactions that includes both Sir1 and Hir1, and possibly other factors yet to be discovered.

The CAF-I and Hir nucleosome assembly proteins have overlapping roles in maintaining high-fidelity chromosome segregation: cells simultaneously lacking *CAC* and *HIR* genes display greatly increased rates of chromosomal nondisjunction (Sharp et al. 2002; Figure 4C). We hypothesized that chromosome stability defects caused by loss of Sir1 would also be exacerbated upon deletion of *CAC* or *HIR* genes. To test this, chromosome missegregation rates were compared in cells with all possible combinations of *sir1*Δ, *hir1*Δ and *cac1*Δ gene deletions (Figure 4C). Consistent with previous data, *cac1*Δ *hir1*Δ cells displayed a 75-fold increase in chromosomal nondisjunction rates. Furthermore, synergistic increases in nondisjunction rates were observed in *sir1*Δ *hir1*Δ and *sir1*Δ *cac1*Δ cells (20-fold and 24-fold, respectively) compared to either wild-type or single mutant cells. Additionally, chromosome nondisjunction rates in *sir1*Δ *cac1*Δ *hir1*Δ triple mutants were statistically indistinguishable from nondisjunction rates of *cac1*Δ *hir1*Δ double mutant cells. Together, these data demonstrate that Sir1 functions in a partially overlapping manner with CAF-I and the Hir proteins to maintain chromosome stability.

In this report, we have demonstrated a novel role for the Sir1 protein in promoting centromeric chromatin structure and function. Sir1 localizes to budding yeast kinetochores and helps maintain mitotic chromosome stability by a mechanism that is independent of interactions known to be critical for forming silent heterochromatin at *HM* loci. These data are consistent with the finding that budding yeast centromeres do not exert position-dependent silencing on neighboring genes (K. Bloom, personal communication), and argue that the role of Sir1 in promoting formation of specialized chromatin structures is not limited to the recruitment of Sir2-4 proteins.

Instead, we suggest that the activity of Sir1 is regulated by chromosomal context. In this

view, protein partners specific to the kinetochore-associated pool of Sir1 form a specialized chromatin structure distinct from that found at heterochromatin. For example, Sir1 copurifies with the kinetochore protein Mcm19 (Nevan Krogan and Jack Greenblatt, personal communication), suggesting that Sir1 may be a peripheral subunit of the outer kinetochore. This concept of chromatin context-dependent function is reinforced by several examples in the literature. First, a budding yeast origin of replication acts as a silencer element only when juxtaposed closely in sequence space to Rap1 and Abf1 binding sites (McNally and Rine 1991; Loo and Rine 1995). Second, the methylation of histone H3 Lys4 is a modification closely associated with active gene transcription, yet is also required for the efficient silencing of RNA polymerase II transcription within rDNA sequences (Briggs et al. 2001; Bryk et al. 2002; Santos-Rosa et al. 2002). Third, the heterochromatin protein HP1 also contributes to inducible gene expression at some euchromatic loci (Piacentini et al. 2003). Identification of Sir1-containing complexes unique to the kinetochore will be an important step in understanding the centromeric function of Sir1.

Previously, we demonstrated that CAF-I and Hir proteins are critical for maintaining the integrity of specialized chromatin structures found at budding yeast kinetochores (Sharp et al. 2002). Here, our data demonstrate a direct physical interaction between the Sir1 protein and the large subunit of Chromatin Assembly Factor-I. Importantly, we found that Sir1 and Hir1 acted together to maintain normal levels of CAF-I at centromeric regions. Also, we determined that Sir1, Hir1 and CAF-I all have overlapping roles in preventing chromosome nondisjunction during mitosis. We propose that an important aspect of the biology of Sir1 at centromeric chromatin is to participate in a structure that acts to recruit CAF-I. We note that although budding yeast has no HP1 homolog, we have discovered that this organism employs an alternative heterochromatin protein, Sir1, to help maintain CAF-I at centromeres. Therefore, diverse eukaryotic organisms use silencing proteins to recruit histone deposition factors to centromeres to support kinetochore function.

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Figure Legends

Figure 1. Association of Sir1 with centromeric chromatin. (A) Presence of Sir1, but not Sir 2-4, at centromeric loci. Formaldehyde cross-linked chromatin prepared from yeast strains CFY416 (*SIR1-HA*, lanes 2 and 4) and PKY346 (*SIR1*, lane 3) was immunoprecipitated in the presence of monoclonal 12CA5 anti-HA antibody (lanes 3-4) or mock treated (lane 2). Chromatin from yeast strains PKY090 (wild-type *SIR2*, *SIR3*, *SIR4*), PKY3342 (*sir2*Δ), PKY3343 (*sir3*Δ), and PKY3344 (*sir4*Δ) was mock treated (lane 6), or immunoprecipitated with antibodies to Sir2 (lanes 7-8), Sir3 (lanes 9-10) and Sir4 (lanes 11-12). PCR was performed to visualize recovery of the core centromeric regions of *CEN3, CEN11,* and *CEN16*, the *HMR-E* silencer, *MAT*, *ACT1*, and two subtelomeric sequences on the right arm of Chromosome VI (*TEL*). Total chromatin was titrated to determine the linear range of the PCR (data not shown); a 1:32 dilution that falls within this range is shown in lanes 1 and 5. **(B) Quantitation of Sir1-4 chromatin immunoprecipitation experiments.** Chromatin immunoprecipitations (n=3 for each genotype) were performed as described in (A). Signal

strength of PCR products was measured using Quantity One software (BIO-RAD) and used to calculate the percent recovery of *HMR-E*, *MAT*, *CEN1-4*, *CEN11*, and *CEN16* PCR products. The average percent recovery is expressed as fold enrichment relative to the *MAT* control locus. **(C) Distribution of Sir1 across the *CEN3* region.** Chromatin was prepared from yeast strain CFY416 (*SIR1-HA*) and PCR was performed as in (Figure 1A). Anti-HA precipitated chromatin, mock-precipitated chromatin, and total chromatin was analyzed for recovery of fragments at or flanking the core centromeric region of *CEN3* as indicated on the diagram (not to scale). **(D) Sir1 colocalizes with centromere protein Ndc10.** Yeast strain PKY2648 (*SIR1-HA*, *NDC10-GFP*) was prepared for indirect immunofluorescece analysis as described (Loidl et al. 1998). Spread nuclei were stained with anti-HA (red), anti-GFP (green) antibodies, and DAPI (blue). Colocalization of Sir1 and Ndc10 is indicated by the yellow staining in the merged image.

Figure 2. Sir1 association with centromeric chromatin requires Ndc10, but is independent of the Orc1 N-terminus, CAF-I, or Hir proteins. Chromatin immunoprecipitation was performed as described in Figure 1. Total (rows 3, 6), mock immunoprecipitations (rows 2, 5) and anti-HA immunoprecipitations (rows 1, 4) were then tested for the presence of *CEN16*, *ACT1*, *HML-E*, *HML-I*, and *MAT* DNA. Yeast strains were PKY2586 (*SIR1-HA*), lanes 1, 6, 7; CFY687 (*sir1*^{R493G}*-HA*), lane 2; CFY689 (*sir1*^{V490D}*-HA*), lane 3; CFY1392 (*SIR1-HA*, *orc1*-NΔ), lane 4; CFY345 (*SIR1*) lane 5; PKY2578 (*SIR1-HA*, *ndc10-1*), lanes 8, 9.

Figure 3. Biochemical interaction between Sir1 and Cac1. (A) Two-hybrid interaction in yeast. Yeast strain PJ69-4a (James et al. 1996) expressed the indicated activation domain (GAD) or DNA binding (GBD) fusion proteins. The GAD-Cac1 plasmid encodes amino acids 217-606 of Cac1, and was recovered in a two hybrid screen using the full-length GBD-Sir1 plasmid. Cells were grown either on media lacking uracil and leucine (-Ura -

Leu) to select for the two plasmids or on -Ura -Leu media also lacking histidine and containing 2.5 mM 3-aminotriazole (-His + 3AT) to score for activation of the *HIS3* reporter gene, indicating interaction between the fusion proteins. **(B) Direct interaction in vitro.** 2.5 μg of unfused GST (lane 6) or GST-Cac1 fusion proteins (Krawitz et al. 2002) (Cac1 amino acids 87-429, lane 2; 87-306, lane 3; 334-429, lane 4; or 429-606, lane 5) pre-bound to 15 μl Glutathione Agarose (Sigma) were incubated with 6 μl ^{35}S-labeled *in vitro* translated Sir1 (Novagen). Reactions were rotated at 4˚C for one hour prior to three one ml washes with Buffer A (25 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% NP-40) + 250 mM NaCl. Bound proteins were eluted with SDS sample buffer, separated by SDS-PAGE, and detected by autoradiography. Lane 1 contains 2μl in vitro translated Sir1 loaded directly onto the gel. **(C) Interaction between CAF-I and Sir1 in cell extracts.** Overproduction of CAF-I including a FLAG-tagged Cac1 subunit and Sir1 in SF9 cells was performed as described (Sharp et al. 2001). 5 μl of nuclear extract containing CAF-I (lane 4), Sir1 (lane 5), or both CAF-I and Sir1 (lane 6) were incubated with 95 μl Buffer A + 50 mM NaCl for two hours at 4˚C in the presence of anti-FLAG antibodies (Sigma) crosslinked to Protein A-Sepharose. Samples were washed three times with one ml Buffer A + 500 mM NaCl. Bound proteins were eluted with SDS sample buffer, separated by SDS-PAGE, and analyzed by immunoblotting with a polyclonal anti-Sir1 antibody. 2.5 μl of total nuclear extract from cells expressing CAF-I alone (lane 1), Sir1 alone (lane 2), or both CAF-I and Sir1 (lane 3) were analyzed on the same gel. The asterisk indicates a crossreacting protein present in the crude cell lysates.

Figure 4. Cac1, Hir1, and Sir1 make overlapping contributions to centromere structure and function. (A) Cac1 association with the core centromeric region requires Sir1 and Hir1. Chromatin immunoprecipitations were performed on yeast strains YB703 (*CAC1-HA*), lane 1; PKY2615 (*CAC1-HA*, *sir1*Δ), lane 2; PKY2617 (*CAC1-HA*, *hir1*Δ), lane 3; and PKY2619 (*CAC1-HA*, *sir1*Δ, *hir1*Δ), lane 4; PKY346 (CAC1), lane 5. Total, mock immunoprecipitations (data not shown), and anti-HA immunoprecipitations were then tested

for the presence of *CEN16* and *ACT1* DNA. A Western blot was performed to compare immunoprecipitation of Cac1-HA from the indicated strains (bottom panel). **(B) Quantitation of Cac1-HA chromatin immunoprecipitation experiments.** Chromatin immunoprecipitations (n=3 for each genotype) were analyzed as described in Figure 1B for the *MAT, CEN3*, and *CEN16* loci. **(C) *SIR1*, *HIR1*, and *CAC1* together prevent chromosomal nondisjunction during mitotic cell divisions.** Nondisjunction rates per cell division were quantified in haploid yeast strains containing a nonessential chromosome fragment by the half-sector colony color assay as described (Shero et al. 1991; Sharp et al. 2002). Yeast strains are PKY847 (wild-type), PKY2609 (*sir1*Δ), PKY2216 (*cac1*Δ), PKY865 (*hir1*Δ), PKY2655 (*sir1*Δ *hir1*Δ), PKY 2610 (*sir1*Δ *cac1*Δ), PKY 2217 (*cac1*Δ *hir1*Δ), and PKY2656 (*sir1*Δ *cac1*Δ *hir1*Δ). Three experiments for each genotype (except PKY2656, n=4) were performed; the average chromosome nondisjunction rate per cell division and standard deviation (error bars) are shown.