# Chl1p, a DNA Helicase-Like Protein in Budding Yeast, Functions in Sister-Chromatid Cohesion

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ABSTRACT

From the time of DNA replication until anaphase onset, sister chromatids remain tightly paired along their length. Ctf7p/Eco1p is essential to establish sister-chromatid pairing during S-phase and associates with DNA replication components. DNA helicases precede the DNA replication fork and thus will first encounter chromatin sites destined for cohesion. In this study, I provide the first evidence that a DNA helicase is required for proper sister-chromatid cohesion. Characterizations of *chl1* mutant cells reveal that *CHL1* interacts genetically with both *CTF7/ECO1* and *CTF18/CHL12*, two genes that function in sister-chromatid cohesion. Consistent with genetic interactions, Chl1p physically associates with Ctf7p/Eco1p both *in vivo* and *in vitro*. Finally, a functional assay reveals that Chl1p is critical for sister-chromatid cohesion. Within the budding yeast genome, Chl1p exhibits the highest degree of sequence similarity to human CHL1 isoforms and BACH1. Previous studies revealed that human CHLR1 exhibits DNA helicase-like activities and that BACH1 is a helicase-like protein that associates with the tumor suppressor BRCA1 to maintain genome integrity. Our findings document a novel role for Chl1p in sister-chromatid cohesion and provide new insights into the possible mechanisms through which DNA helicases may contribute to cancer progression when mutated.

PROPER transmission of the parental genome requires that chromosomes are first replicated and that the resulting sister chromatids are faithfully segregated away from each other into the newly forming daughter cells. From the time of chromosome replication until chromosome segregation, sister chromatids are paired together. This pairing, or sister-chromatid cohesion, enables the cell to identify over time the products of DNA replication as sisters. In addition, cohesion ensures that one chromatid associates with microtubules from the spindle pole opposite that of its sister chromatid. Only at anaphase onset is cohesion inactivated, allowing one chromatid to move away from its sister along the mitotic spindle apparatus (Koshland and Guacci 2000; Nasmyth *et al.* 2000).

In budding yeast, several classes of cohesion factors have been identified. Structural cohesion proteins (cohesins) maintain sister-chromatid cohesion from DNA replication until anaphase onset. Structural cohesins include Smc1p, Smc3p, Mcd1p/Scc1p, Irr1p/Scc3p, and Pds5p (Strunnikov *et al.* 1993; Kurlandzka *et al.* 1995; Guacci *et al.* 1997; Michaelis *et al.* 1997; Toth *et al.* 1999; Hartman *et al.* 2000; Panizza *et al.* 2000). Deposition cohesion factors include Scc2p (Mis4p in fission yeast) and Scc4p, which form a complex separate from the cohesins. The Scc2p,Scc4p deposition complex is thought to load the structural cohesins onto chromatin (Furuya *et al.* 1998; Toth *et al.* 1999; Ciosk *et al.* 2000). Establishment factors such as Ctf7p/Eco1p are required to couple the processes of cohesion and DNA replication to ensure that only sister chromatids become paired together (Skibbens *et al.* 1999; Toth *et al.* 1999). In contrast to the structural cohesins, the deposition and establishment factors are required only during S-phase. More recently, several DNA replication factors have been identified as functioning in cohesion, including replication factor C (RFC) subunits and DNA polymerases, cementing the link between cohesion and DNA replication (Wang *et al.* 2000; Hanna *et al.* 2001; Mayer *et al.* 2001; Edwards *et al.* 2003; Kenna and Skibbens 2003).

Presently, the establishment of cohesion is poorly understood. Certainly, loss of cohesion establishment factors such as Ctf7p/Eco1p (herein termed Ctf7p) leads to precocious sister-chromatid separation and cell death. However, Ctf7p is not required to maintain cohesion nor deposit cohesins onto DNA. Instead, budding yeast Ctf7p appears to establish cohesion in part by coupling the cohesion machinery to DNA replication by directly interacting with the replication machinery. The finding that Ctf7p is an acetyltransferase provided an important clue and suggested a model in which cohesion establishment occurs by chromatin remodeling near the DNA replication fork (Skibbens *et al.* 1999; Toth *et al.* 1999; Ivanov *et al.* 2002).

Similar to *CTF7*, budding yeast *CHL1* was also identified by virtue of decreased chromosome transmission fidelity or chromosome loss screens. Mutations in *CHL1*

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result in increased chromosome loss, sister-chromatid nondisjunction, and a variety of phenotypes, including bisexual mating of diploids, donor locus selection defects in *MAT***a** cells, and increased mitotic recombination (Haber 1974; Liras *et al.* 1978; Gerring *et al.* 1990; Spencer *et al.* 1990; Weiler *et al.* 1995). In combination, these findings suggested that Chl1p is critical for higher-order chromatin conformations that, in addition to blocking inappropriate recombination, are central to chromosome segregation (Weiler *et al.* 1995). Chl1p exhibits significant homology to Rad3p, a DNA helicase that exhibits nucleotide excision repair activity (Gerring *et al.* 1990). Human CHLR1 protein exhibits DNA helicase activity, binding both single- and double-stranded DNA (Amann *et al.* 1997; Hirota and Lahti 2000). In this report, I find that budding yeast Chl1p physically associates with Ctf7p and also provide functional analyses that Chl1p is critical for sister-chromatid cohesion. The combination of these findings both documents a new role for Chl1p in sister-chromatid cohesion and provides insight into the mechanisms through which DNA helicases contribute to genome stability.

## MATERIALS AND METHODS

**Media and cell growth and database methods:** Growth and sporulation media for yeast were described previously (Ito *et al.* 1983; Rose *et al.* 1990). Dissections and growth involving *ctf7* (temperature sensitive) were performed at 25°; those involving *pol30* or *ctf18* (both are cold sensitive) were performed at 30° unless otherwise indicated. Yeast and bacterial transformations were performed as described with minor modifications (Ito *et al.* 1983; Schiestl and Gietz 1989). S288C-derived *Saccharomyces cerevisiae* yeast strains (YPH) and plasmids (pRS) were described previously (Sikorski and Hieter 1989; Gerring *et al.* 1990; Doheny *et al.* 1993).

Budding yeast Ctf7p and Rad3p amino acid sequences and human BACH1 amino acid sequence were used to perform reciprocal BLAST searches (BLASTP, version 2.2.5) using default parameters (Altschul *et al.* 1997) of protein sequence databases in yeast (http://www.ncbi.nlm.nih.gov/BLAST/Genome/YeastBlast.html) and recent submissions to the human genome project (http://www.ncbi.nlm.nih.gov/BLAST).

**Molecular methods and epitope tagging:** A PCR strategy was used to generate yeast cells in which Chl1-13MYCp was the sole source of Chl1p function. Briefly, a 1.7-kb sequence encoding for the C-terminal *CHL1* open reading frame was obtained using *Xho*I and *Bgl*II and cloned into pRS306-Δ*Xba*I digested with *Xho*I and *Bam*HI. To ensure improper transcription of epitope-tagged *CHL1* after integration, a frameshift was generated via *Cla*I digestion, fill in, and religation. PCR and oligos AAGAATTCTTCGTACGCTGCAGGTCGACGG and CATAAGAAATTCGCTTATTTAGAAGTGG were then used to generate a DNA fragment containing 13 MYC epitope coding sequences (Longtine *et al.* 1998) flanked by *Eco*RI sites. The resulting PCR product and pRS306-Δ*Xba*I-*CHL1*$^{3'\ \text{region}}$ were digested with *Eco*RI and ligated together, placing the 13 MYC epitope in frame with the *CHL1*$^{\text{C terminus}}$. The resulting plasmid was linearized with *Xba*I, residing within the *CHL1* open reading frame, and integrated by transformation into YPH499 (Sikorski and Hieter 1989). Integration/tagging of *CHL1*, producing YBS1129, was confirmed using PCR, media selection, and Western blot analyses.

**Immunofluorescence and Western blot analyses:** Flow cytometry, Western blot analysis, and indirect immunofluorescence were performed as previously described (Gerring *et al.* 1990; Cohen-Fix *et al.* 1996; Skibbens *et al.* 1999) with minor modifications. Immunostainings/immunodetections were performed using the anti-hemagglutinin (anti-HA) 12CA5 (BabCo), anti-MYC 9E10 (Santa Cruz), or anti-MYC B-14 (Santa Cruz) antibodies in combination with goat anti-mouse HRP (Bio-Rad, Richmond, CA) antibody, goat anti-mouse ALEXA (Molecular Probes, Eugene, OR), or goat anti-rabbit ALEXA (Molecular Probes) antibodies and enhanced chemiluminescence (ECL; Amersham-Pharmacia) for visualization.

**Co-immunoprecipitations:** Co-immunoprecipitations were performed as previously described with minor modifications (Lamb *et al.* 1994). Briefly, log phase Chl1-13MYCp strains coexpressing Ctf7-HAp (pBS9 episome) were lysed via bead beating (Biospec Products, Bartlesville, OK), pelleted, and the supernatant incubated with anti-cMYC 9E10 antibody (Santa Cruz) and protein A Sepharose beads (Pharmacia, Piscataway, NJ). The beads were copiously washed in ELB (120 mm NaCl, 50 mm HEPES, pH 7.6, 5 mm EDTA) supplemented with protease inhibitors (Roche) and the bound proteins removed with SDS Laemmli buffer. Chl1p and Ctf7p were visualized using anti-MYC 9E10 (Santa Cruz), anti-HA Y-11 (Santa Cruz), followed by goat anti-mouse HRP or goat anti-rabbit HRP (Bio-Rad) antibody and ECL-*Plus* (Amersham-Pharmacia) for visualization.

**GST pull-downs:** Glutathione *S*-transferase (GST) pull-downs were performed as previously described with the following modifications (Kenna and Skibbens 2003). Briefly, yeast strains expressing either MYC-tagged or untagged Chl1p were spheroplasted in 100T Zymolyase (Seikagaku, Rockville, MD) and lysed by swelling (20 mm HEPES-HCl pH 7.5, 5 mm $MgCl_2$ + protease inhibitors). Whole-cell extracts were then centrifuged at 9500 rpm for 45 min (Beckman JA-20) and the clarified supernatants containing soluble proteins were harvested (Bogerd *et al.* 1994). Supernatants were then incubated with glutathione Sepharose beads coupled previously to either GST or GST-Ctf7p (Kenna and Skibbens 2003). After incubation with supernatants, beads were washed several times before eluting bound proteins. Western blot analyses for MYC-tagged protein were performed using a monoclonal anti-cMYC 9E10 antibody (Santa Cruz) followed by goat anti-mouse HRP (Bio-Rad) antibody and ECL-*Plus* (Amersham-Pharmacia) for visualization.

**Cohesion assays:** Defects in cohesion were assessed in two chl1 null strains independently derived. In the first case, we used a strain in which *HIS3* integration within the *CHL1* open reading frame generated a *chl1* disruption allele (Gerring *et al.* 1990). This *chl1* disruption strain (YPH698) was crossed to our cohesion assay strain YBS1045 (*tetO:URA3*, *tetR-GFP:LEU2* and *PDS1-13MYC:TRP*) previously described (Gerring *et al.* 1990; Kenna and Skibbens 2003). The resulting diploids were sporulated and dissected, and progeny containing the appropriate markers (Ura+, His+, Trp+, and Leu+) were identified as YBS1125. In the second case, the entire *CHL1* open reading frame was precisely replaced with *KAN* using a PCR strategy (Longtine *et al.* 1998) and oligos GTAGAAAACCAG GCTAAAAACAGTCACACTAGTCCAAAAACGGATCCCCGG GTTAATTAA and ATATAGTAGTAATCACAGTATACACGT AAACGTATTCCTTGAATTCGAGCTCGTTTAAAC. Correct integration/replacement into our cohesion assay strain (YBS1045) was confirmed by multiple PCR reactions and resistance to G418-containing media to produce YBS1142. Cohesion assays on the above strains were performed as described previously (Kenna and Skibbens 2003). Briefly, log-phase wild-type and *chl1* null cells were arrested in mitosis using

**TABLE 1**

**Parents and products of crosses**

| | Observed | Expected |
|---|---|---|
| *ctf7-203:LEU2, ctf7::HIS3 × chl1Δ::TRP1* | | |
| Wild type | 15 | 15 |
| *chl1* (Trp+) | 10 | 15 |
| *ctf7* (Leu+, His+, ts) | 13 | 7.5 |
| *ctf7, chl1* | 1[a] | 7.5 |
| Inviable[b] | 21 | 15 |
| *chl1Δ::TRP1 × ctf18Δ::URA3* | | |
| Wild type | 24 | 25 |
| *chl1* | 24 | 25 |
| *ctf18* | 19 | 25 |
| *chl1, ctf18* | 0 | 25 |
| Inviable[b] | 32 | 0 |
| *chl1Δ::TRP1 × pol30-104:LEU2* | | |
| Wild type | 21 | 20 |
| *chl1* | 16 | 20 |
| *pol30* | 16 | 20 |
| *chl1, pol30* | 23 | 20 |
| Inviable[b] | 4 | 0 |

[a] Scoring of dissection plates replica plated to selection plates after 1 day of growth. A second *chl1, ctf7* double-mutant spore was identified after an additional 3 days of incubation.

[b] Number of spores expected in the absence of a synthetic lethal genetic interaction.

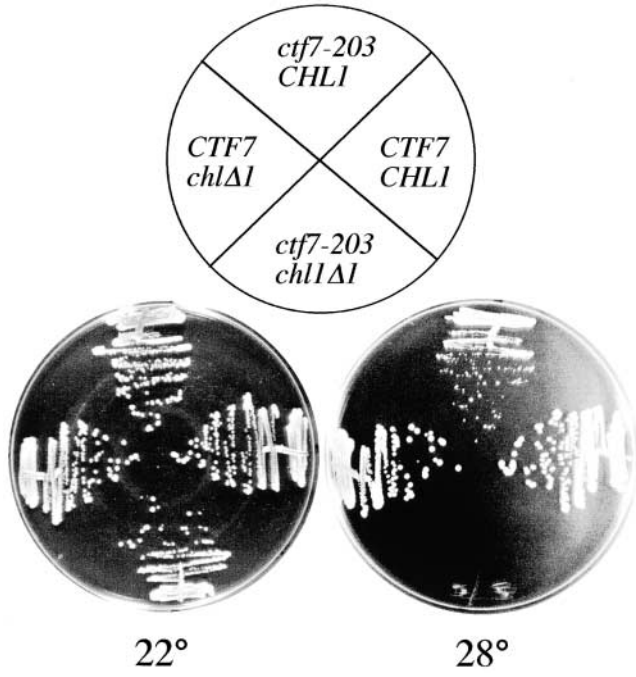


FIGURE 1.—*CHL1* and *CTF7* genetically interact. A *chl1* null allele exhibits conditional synthetic lethality when combined with *ctf7-203*. Plates of wild-type, *ctf7-203*, *chl1* null, and *ctf7-203-chl1* double-mutant strains grown at both 22° and 28° are shown.

nocodazole, fixed, and processed for immunofluorescence to view Pds1p, DNA (DAPI), and chromatid loci (GFP) and for flow cytometry to assess DNA content. Figure 5C represents data tallied from three separate experiments.

## RESULTS

***CHL1* genetically interacts with *CTF7* and *CTF18*:** The role of Ctf7p in establishing cohesion between sister chromatids during S-phase is now firmly established (SKIBBENS *et al.* 1999; TOTH *et al.* 1999). More recent evidence that Ctf7p exhibits acetyltransferase activity suggests that cohesion establishment may be coupled to chromatin remodeling during DNA replication (IVANOV *et al.* 2000). On the basis of published phenotypes of *chl1* mutant strains, a likely role for Chl1p is to mediate the assembly of chromatin structures during DNA replication (LIRAS *et al.* 1978; GERRING *et al.* 1990; LI and MURRAY 1991; WEILER *et al.* 1995; HOLLOWAY 2000). I decided to search for genetic interactions to first test whether the roles of Chl1p and Ctf7p were related. Yeast strains harboring the temperature-sensitive *ctf7-203* mutation were crossed with strains in which *CHL1* was disrupted by the *TRP1* locus. The resulting diploids were sporulated and cells harboring both the *ctf7-203* allele and *chl1* null mutations were identified. Of 60 possible spores, only 40 spores were viable and gave rise to colonies. Of these 40 viable strains, 1 contained both *ctf7-203* and *chl1* null mutations (a second spore harboring both mutations was identified after several days of additional growth). This frequency of recovering double-mutant strains is significantly below expected, revealing that *ctf7* and *chl1* genetically interact (Table 1). To further assess this genetic interaction, yeast strains harboring mutations in *ctf7*, *chl1*, or both *ctf7* and *chl1* (obtained from the above crosses) were placed on YPD-rich plates and incubated at either 22° or 28°. After several days of growth, both *ctf7* and *chl1* single-mutant strains exhibited robust growth at 22° and 28°. The *ctf7-chl1* double-mutant strain also exhibited growth at 22°. In contrast to the other strains, however, the *ctf7-chl1* double-mutant strain was inviable at 28° (Figure 1). These results indicate that *ctf7* and *chl1* exhibit a conditional synthetic lethal interaction.

A previous study revealed that alleles of *CTF7* are synthetically lethal when combined with a null mutation of *CTF18* (also called *CHL12*, which encodes for an RF-C homolog; KOUPRINA *et al.* 1994; SKIBBENS *et al.* 1999). Subsequent work revealed that Ctf18p plays an important but nonessential function in cohesion establishment (HANNA *et al.* 2001; MAYER *et al.* 2001). Given the conditional synthetic lethal interaction between *ctf7* and *chl1*, I next tested for genetic interactions between *chl1* and *ctf18*. Strains harboring loss-of-function alleles for either *CHL1* or *CTF18* were crossed and the resulting diploid strains were sporulated and dissected to obtain individual spore progeny (MATERIALS AND METHODS). Of 100 possible spores, 68 spores were viable. The num-

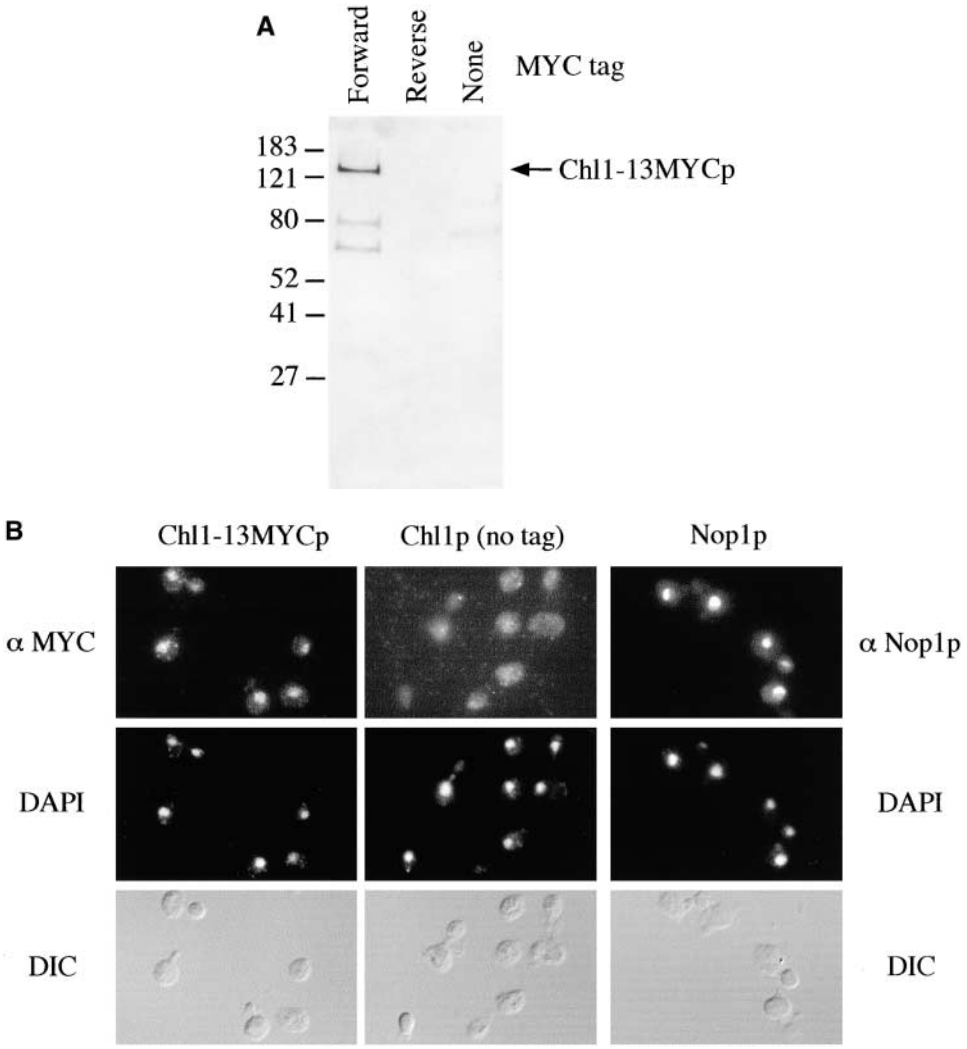


FIGURE 2.—Expression and localization of Chl1p. (A) MYC-specific antibodies recognize a protein band of the appropriate molecular weight from cell lysates expressing Chl1-13MYCp as the sole source of Chl1p function (Forward). Similar protein bands were not detected in cells expressing 13 MYC epitopes fused in the reverse orientation (Reverse) or in untagged cells (None). Protein bands of ~80 kD and 65 kD are consistent with Chl1-13MYCp breakdown products. (B) Chl1p localizes to the nucleus. Micrographs of wild-type and epitope-tagged Chl1p strains in which Chl1-13MYCp was visualized using MYC-specific antibodies (αMYC). In most cells, Chl1p is completely coincident with DAPI staining (DAPI). In comparison, Nop1p is limited to crescent-shape nucleolar regions (αNop1p) adjacent to but separate from the bulk of the nucleus. Cell structures by differential interference contrast microscopy (DIC) are also shown.

ber of single-mutant *chl1* or *ctf18* strains recovered matched the number of single-mutant spores expected, indicating that loss of either Chl1p or Ctf18p function did not adversely affect sporulation or germination (Table 1). In contrast, no spores were recovered that contained both *chl1* and *ctf18* mutations. These results suggest that loss of both Chl1p and Ctf18p activities is lethal. In summary, these findings document genetic interactions between *CHL1* and two genes that are critical for sister-chromatid cohesion, *CTF7* and *CTF18*.

Alleles of *CTF7* are also synthetically lethal when combined with alleles of *POL30* (encodes for proliferating cell nuclear antigen, or PCNA; BAUER and BURGERS 1990; SKIBBENS *et al.* 1999). Given the genetic interaction between *CTF7* and *CHL1*, I decided to also test for a genetic interaction between *CHL1* and *POL30*. *chl1* null cells were crossed to *pol30-104* cells and the resulting diploid strains were sporulated and dissected to obtain individual spore progeny. Of 80 possible spores, 76 spores were viable, resulting in 95% viable progeny (Table 1). Moreover, 23 *chl1-pol30-104* double-mutant spores were recovered, compared to the 20 expected. These findings indicate that *CHL1* does not exhibit a synthetic lethal interaction with *POL30* (at least for the *pol30-104* allele tested). More importantly, these findings substantiate that the genetic interactions reported above for *CHL1*, *CTF7*, and *CTF18* are specific and not due to a general decrease of S-phase factor activities.

**Chl1p is a nuclear protein:** Previous cell fractionation studies suggested that Chl1p is a nuclear protein (HOLLOWAY 2000). However, direct observation of Chl1p localization has never been successfully performed, possibly due to low expression levels of Ch1lp. Complicating the issue is the finding that human CHLR1 is a nucleolar protein (AMANN *et al.* 1997). I decided to address the localization of Chl1p in budding yeast. To facilitate localization of Chl1p *in vivo*, I generated yeast strains in which 13 MYC-tagged Chl1p were the sole source of Chl1p function (MATERIALS AND METHODS). PCR and Western blot analyses confirmed the correct integration and expression of Chl1-13MYCp (Figure 2A). To localize Chl1p within yeast cells, log-phase wild-type and Chl1-13MYCp-expressing cells were processed for immunofluorescence and Chl1p was visualized using MYC-

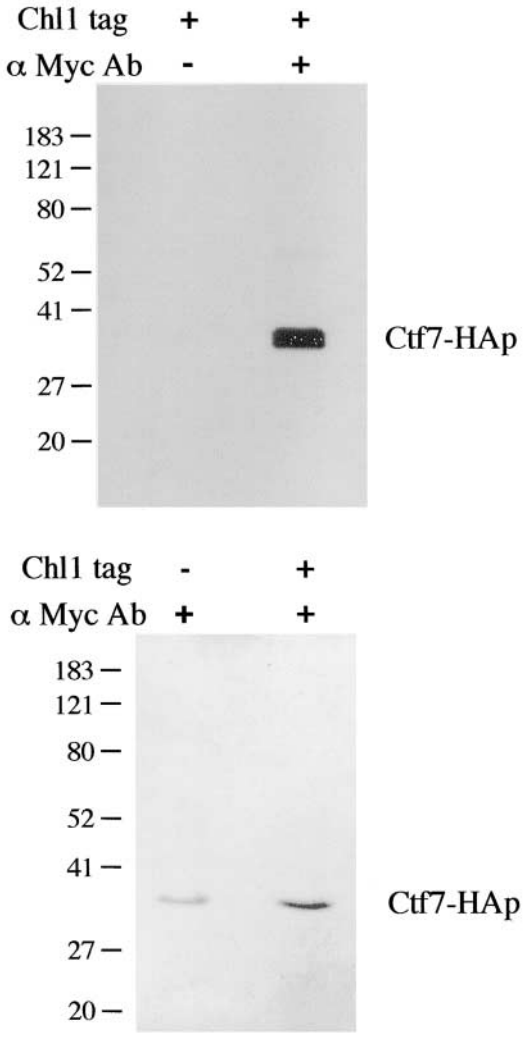


FIGURE 3.—Chl1p and Ctf7p physically associate *in vivo*. (Top) Cell lysates coexpressing Chl1-13MYCp and Ctf7-HAp were incubated with or without MYC-directed antibody. Associated proteins were then precipitated with protein A Sepharose beads. After several washes, the beads were eluted and Ctf7p co-immunoprecipitation assayed by Western blot using HA-directed antibody. (Bottom) MYC-tagged *vs.* untagged Chl1p cell lysates, both containing Ctf7-HAp, were treated with MYC-directed antibody and protein A Sepharose beads. After several washes, the beads were eluted and Ctf7p co-immunoprecipitation assayed by Western blot as above.

specific antibodies. For comparison, localization of the nucleolar protein Nop1p was also assessed (ARIS and BLOBEL 1988). In the majority of cells, Chl1p signal was completely coincident with that of a DNA intercalating dye 4′,6-diamidino-2-phenylindole (DAPI), indicating that Chl1p localizes to the bulk of the nuclear volume (Figure 2B). A similar nuclear signal was absent in untagged wild-type cells (occasional background speckles were observed). In contrast, Nop1p visualization produced the characteristic crescent-shape nucleolar structure that is adjacent to but separate from DAPI staining (DAPI exhibits an intense signal in the bulk of the nucleus but is greatly reduced or absent from the nucleolus; see Figure 2B). Despite the use of highly preabsorbed secondary antibodies, cross-reactivities rendered colocalization studies in the same cell impractical. These findings provide the first evidence regarding *in vivo* yeast Chl1p localization and are consistent with a role for Chl1p in chromosome segregation.

**Chl1p physically associates with Ctf7p:** Often a genetic interaction reflects a physical association between two proteins. Given the conditional synthetic lethal interaction between *CHL1* and *CTF7* mutants, I tested for a physical association between Chl1p and Ctf7p *in vivo* using co-immunoprecipitation methods. Extracts from log-phase yeast cells containing Chl1-13MYCp as the sole source of Chl1p function were centrifuged and the supernatant fraction harvested. However, antibodies directed against either endogenous Ctf7p or epitope-tagged Ctf7p expressed at endogenous levels failed to detect Ctf7p, suggesting that Ctf7p occurs at extremely low levels in the cell. To circumvent this problem, a Ctf7-HAp construct that expresses Ctf7p at elevated levels was first transformed into tagged and untagged Chl1p strains. Ctf7-HAp is fully functional in that overexpressed Ctf7-HAp maintains viability of *ctf7*Δ cells at wild-type growth rates (SKIBBENS *et al.* 1999).

To test whether Ctf7p co-immunoprecipitates with Chl1p, soluble Chl1-13MYCp-containing lysates were incubated with and without MYC-directed antibodies, followed by incubation with protein A Sepharose beads (MATERIALS AND METHODS). The beads were washed several times prior to eluting bound proteins. Western blot analyses reveal that Chl1p-13MYCp is immunoprecipitated in the presence of the MYC-directed antibody. I then probed Western blot membranes containing the immunoprecipitated fractions using HA-directed antibodies. The results show that Ctf7p co-immunoprecipitates with Chl1p (Figure 3). While overexposure reveals that a portion of Ctf7p also associates with beads in the absence of antibody, this quantity, if greatly reduced, compared to the amount of Ctf7p bound to Chl1p-antibody-bead complexes. As an additional control, I also tested for Ctf7p co-immunoprecipitation in the presence of MYC-directed antibodies and beads but using lysate produced from untagged and tagged Chl1p cells. Again, Ctf7p co-immunoprecipitated with Chl1p-13MYCp. Ctf7p also associated, but at reduced levels, with MYC antibody-Sepharose bead complexes (Figure 3). Thus, both the MYC antibody and MYC-tagged Chl1p were required for maximal Ctf7p co-immunoprecipitation. These findings reveal that Chl1p and Ctf7p physically associate *in vivo*, linking DNA helicase to cohesion establishment activities near the DNA replication fork.

I next used GST-based chromatography to test independently whether Chl1p and Ctf7p would associate *in vitro* and in the absence of *in vivo* assembly reactions. The entire *CTF7* open reading frame was inserted, in frame, behind GST. Western blot analysis of *Escherichia*

*coli* cells expressing this construct (GST-Ctf7p) identify a plasmid-dependent band of the appropriate molecular weight (Kenna and Skibbens 2003). Bacterially expressed GST-Ctf7p or GST alone (as a control) were then bound to glutathione Sepharose beads, followed by several washes to remove unbound proteins. In parallel, yeast extracts harboring Chl1-13MYCp were subjected to centrifugation to generate a clarified supernatant containing soluble proteins (Bogerd *et al.* 1994). The Chl1p-clarified supernatant (load) was then incubated with GST or GST-Ctf7p bead matrices. The beads were then washed and bound proteins eluted. Western blot analyses of the eluants reveal that Chl1p bound specifically to GST-Ctf7p (Figure 4). In contrast, only trace amounts of Chl1p, when detectable at all, were found to associate with GST alone. These results indicate that, *in vitro*, Chl1p associates specifically with Ctf7p as a soluble complex.

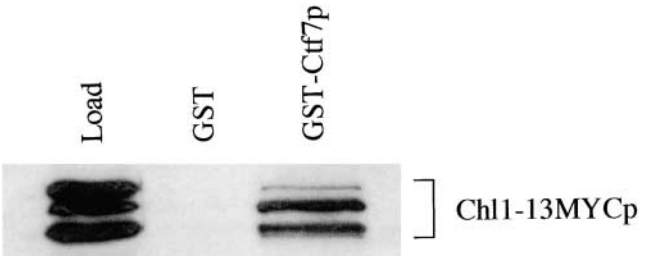


Figure 4.—Chl1p and Ctf7p physically associate *in vitro*. Clarified Chl1-13MYCp supernatants (Load) were incubated with glutathione Sepharose beads coupled to either bacterially expressed GST or GST-Ctf7p. The beads were washed and bound proteins eluted. The ability of soluble Chl1p to associate *in vitro* with GST-Ctf7p, but not GST alone, was then assayed by Western blot using MYC-directed antibodies. Chl1-13MYCp breakdown products are also visible.

**Chl1p functions in sister-chromatid cohesion:** Given the physical association of Chl1p and Ctf7p, a likely model was that Chl1p plays a key role in cohesion establishment. To test this model directly, two unique *chl1* loss-of-function alleles (materials and methods) were introduced into a cohesion assay strain (Kenna and Skibbens 2003). In this assay strain, Tet operator repeats (*TetO*) are integrated at *URA3*, ~40 kb from the centromere of chromosome V. Expression of green fluorescent protein (GFP)-tagged *Tet* repressor protein (*TetR-GFP*) in turn allows for visualization of the centromere-proximal locus (Michaelis *et al.* 1997). Visualization of the GFP signal was then used to determine the position of one sister chromatid relative to the other in both wild-type and *chl1* mutant cells. To verify that cells were arrested prior to anaphase onset, I also performed indirect immunofluorescence to visualize Pds1p. Pds1p is a biochemical marker for pre-anaphase cells (Cohen-Fix *et al.* 1996). Following this regime, cell morphology, GFP-tagged chromosomal loci, and epitope-tagged Pds1p were simultaneously assessed on a cell-by-cell basis, allowing us to map each cell within the cell cycle and assess the disposition of sister-chromatid loci in pre-anaphase cells.

To assay for cohesion defects, log-phase *chl1* mutant and wild-type marker strains were placed in media supplemented with nocodazole to inhibit anaphase onset. After ~2 hr growth at 30°, parallel cell samples were harvested and prepared to assess DNA content, cell morphology, Pds1p content, and disposition of sister-chromatid loci via GFP (see materials and methods). As expected, both wild-type and *chl1*Δ cells treated with nocodazole were predominantly large budded and contained a 2C DNA content, indicative of a mitotic arrest (Figure 4). Cells that retained Pds1p staining that was coincident with DAPI staining, indicative of pre-anaphase cells, were then assessed for cohesion. When GFP-tagged loci were viewed by epifluorescent microscopy, wild-type cells were found to contain tightly paired sister chromatids such that few (12%) sisters were dissociated. In contrast, *chl1*Δ mutant cells contained a significant increase in the number of separated sisters (23%; Figure 5). This level of cohesion defect (23%) is similar to those exhibited by other nonessential cohesion factors (*trf4* at 20%, *ctf18* at 25%, *ctf8* at 30%, and *ctf18* at 35%; Wang *et al.* 2000; Hanna *et al.* 2001; Mayer *et al.* 2001). Both wild-type and *chl1*Δ strains exhibited similarly low levels (~0–2%) of separated sisters in $G_1$ cells arrested using α-factor, indicating that the increase of cells harboring two GFP spots (sister loci) in mitotic *chl1*Δ mutant cells was not due to aneuploidy present early in the cell cycle. A similar role for Chl1p in sister-chromatid cohesion has been independently identified, confirming the results above (M. Mayer, I. Pots and P. Hieter, personal communication).

**Budding yeast Chl1p exhibits the highest level of homology to human BACH1:** As previously described, budding yeast Chl1p exhibits significant sequence similarity to human CHL1 isoforms (Amann *et al.* 1997). In contrast, the budding yeast Sgs1p RecQ helicase exhibits sequence similarity to several human DNA helicases, including those involved in Bloom's and Werner syndromes (Brosh and Bohr 2002; Thompson and Schild 2002), suggesting that Chl1p may have extended but as-yet-unreported homologies. To determine if budding yeast Chl1p exhibits homology to a human DNA helicase other than hCHL1 isoforms, I performed computer-assisted searches using budding yeast Chl1p to query human sequence databases. As expected, human hCHL1-related proteins exhibited significant similarity to yeast Chl1p (*P* values ranging from 6*e*-67 to 1*e*-117). In addition, however, I found that human BACH1 also exhibited a significant level of homology to budding yeast Chl1p (*P* value of 8*e*-58; Figure 6). BACH1 is a helicase-like protein that interacts directly with the tumor suppressor BRCA1 (Cantor *et al.* 2001). BACH1 is highly conserved through evolution, and similarity to budding yeast Rad3p has been reported previously (Cantor *et al.* 2001). To test whether budding yeast Chl1p or Rad3p

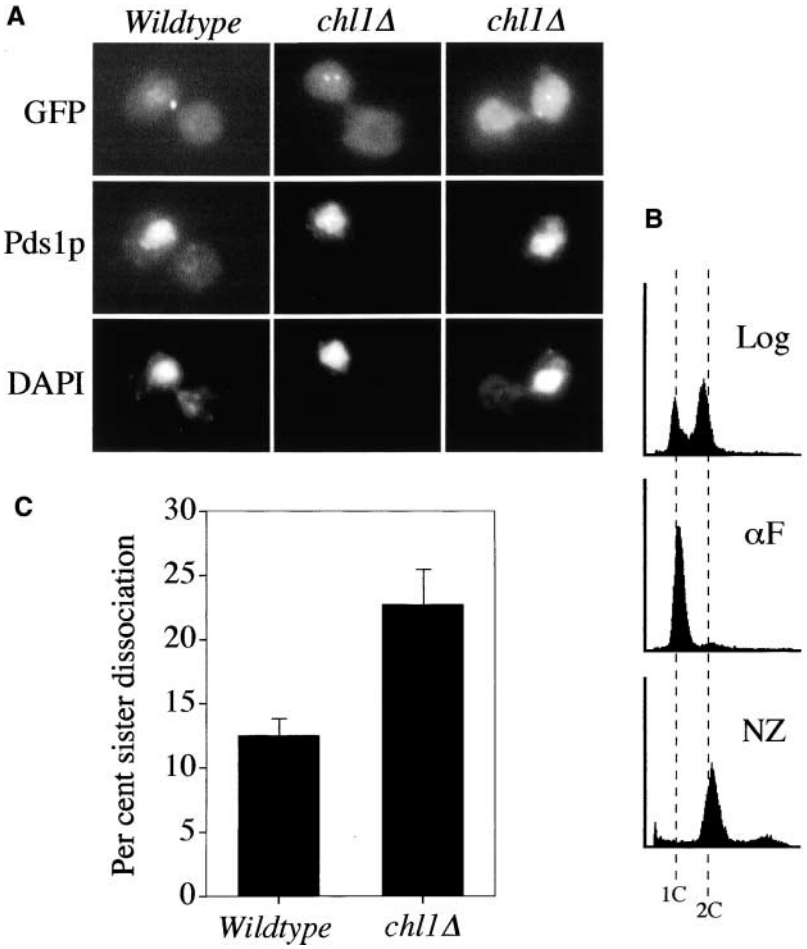


FIGURE 5.—Chl1p functions in sister-chromatid cohesion. (A) Micrographs of *chl1* and wild-type cells showing sister-chromatid loci (GFP), Pds1p (Pds1p), and DNA (DAPI). (B) DNA content, in addition to cell morphology and Pds1p staining, was used to map cells within the cell cycle. Disposition of sister chromatids was determined in both α-factor-arrested (αF) $G_1$ cells and nocodazole-treated (NZ) pre-anaphase cells. Flow cytometer profiles for the cell cycle states were nearly identical for wild-type and *chl1* cells (*chl1* cells are shown). (C) The average percentage of dissociated sisters obtained from three different experiments and two independent *chl1* mutant cells is shown (error bars represent standard deviation).

exhibited a higher degree of similarity to BACH1, both yeast sequences were used to query the human sequence database. As reported, I found that Rad3p exhibits significant sequence similarity to human BACH1 (*P* value of 1*e*-42). However, Chl1p exhibits even a greater level of sequence similarity to human BACH1 (*P* value of 8*e*-58).

To explore further the conservation of human BACH1 within the budding yeast genome, I performed a reciprocal computer-assisted search in which the human BACH1 sequence was used to identify yeast proteins. Consistent with the above findings, I found budding yeast Chl1p as the highest ranking yeast protein that exhibits significant sequence similarity to human BACH1 (*P* value of 3*e*-58). Following this best-fit homology of Chl1p is Rad3p, which exhibited a significant, but reduced, level of similarity (*P* value of 5*e*-43). Interestingly, the extensive sequence similarity exhibited between human BACH1 and yeast Chl1p occurs along the entire open reading frame. In contrast, an ~200-amino-acid segment is absent in budding yeast Rad3p, which corresponds to human BACH1 residues 65–263 (Figure 6). These missing Rad3p residues correspond to helicase domain IA and to a putative nuclear localization signal (CANTOR *et al.* 2001).

## DISCUSSION

Characterization of *CHL1* mutant yeast strains reveals strong genetic interactions when combined with either *CTF7* or *CTF18* mutations. Ctf7p is an essential yeast protein that functions during S-phase to establish sister-chromatid cohesion (SKIBBENS *et al.* 1999; TOTH *et al.* 1999). Consistent with an S-phase activity, Ctf7p associates with each of three sliding clamp loading complexes composed of Rfc2p–Rfc5p in combination with Rfc1p, Rad24p, or Ctf18p (KENNA and SKIBBENS 2003). Several of these factors, including Ctf18p, have been shown to function in sister-chromatid cohesion (HANNA *et al.* 2001; KRAUSE *et al.* 2001; MAYER *et al.* 2001; KENNA and SKIBBENS 2003). One possibility is that the genetic interactions observed between *CHL1*, *CTF7*, and *CTF18* are nonspecific and instead are based on additive defects of S-phase factors. Several findings refute this model. First, *CHL1* exhibits strong genetic interactions with *CTF7* and *CTF18*, both of which function in sister-chromatid cohesion. Second, *CHL1* does not exhibit synthetic lethal interactions when combined with mutations in *POL30*. *POL30* encodes for the sliding clamp PCNA that is essential for DNA replication and loaded onto double-stranded DNA by RFC complexes (KELMAN 1997). Third, co-immunoprecipitation and GST pull-

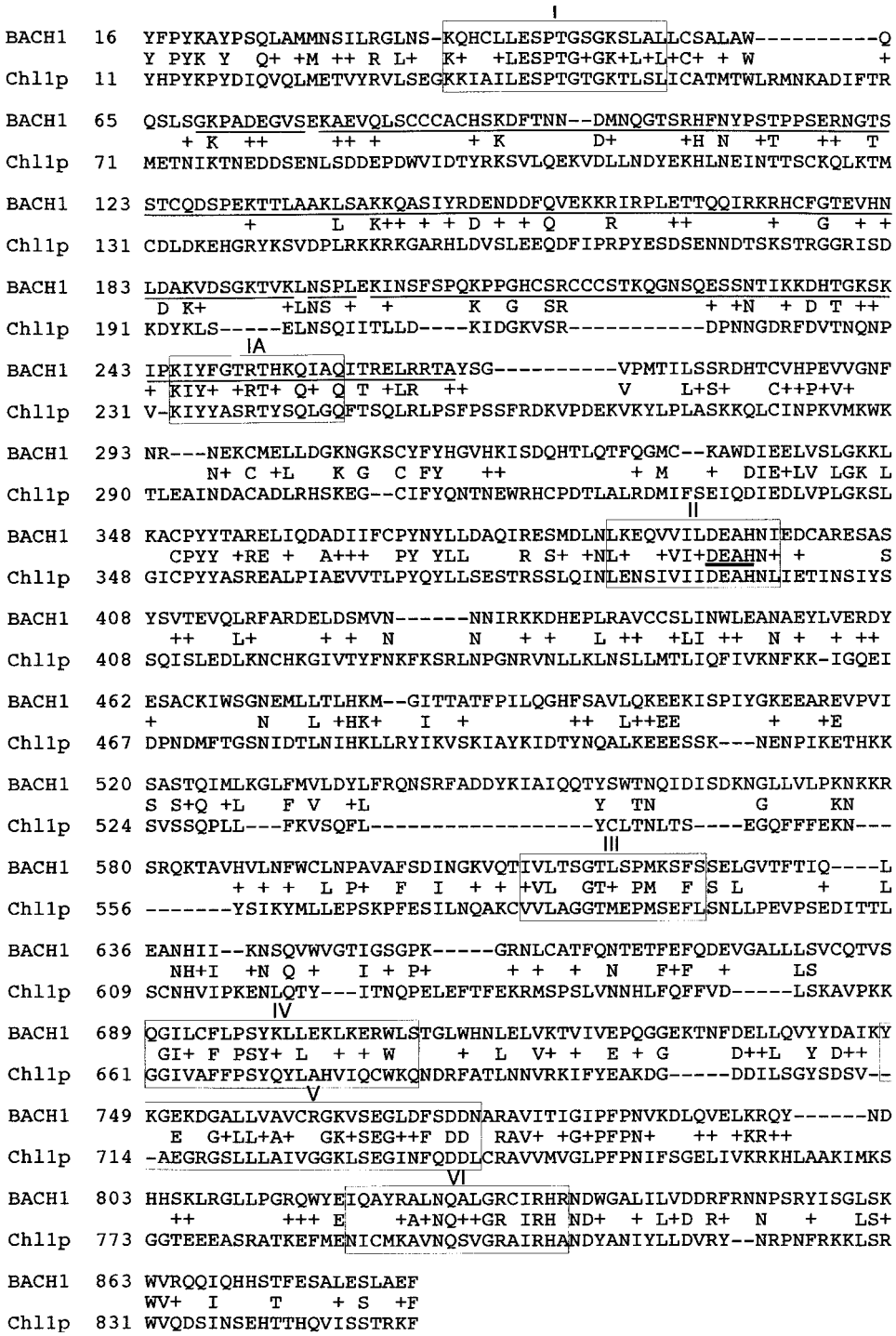


FIGURE 6.—Sequence alignment for budding yeast Chl1p and human BACH1. Budding yeast Chl1p is 24% identical and 42% similar to human BACH1 ($P$ value of $3e$-58). The ~200-residue BACH1 region absent from yeast Rad3p is underlined in the BACH1 sequence. DEAH helicase homology regions are boxed and denoted by roman numerals (based on CANTOR *et al.* 2001). The DEAH motif is underlined in the consensus sequence. Significant sequence similarities (not shown) were also noted between budding yeast Chl1p and the helicase-like protein NHL, a tumor necrosis factor receptor superfamily member ($P$ value $7e$-25) and *Xeroderma pigmentosum* group D complementing protein ($P$ value $1e$-24).

down studies reveal that Chl1p and Ctf7p physically associate *in vivo* and *in vitro*, providing a molecular basis for the genetic interaction observed between *CHL1* and *CTF7*. This association is consistent with those previously reported: that Ctf7p associates with a subset of DNA replication factors in the absence of DNA (KENNA and SKIBBENS 2003). Thus, *CHL1* genetically interacts in a physiologically relevant manner with two S-phase-specific cohesion factors encoded by *CTF7* and *CTF18* (on the basis of RFC homology), but not with another S-phase factor encoded by *POL30*.

In this report, I also provide direct evidence that Chl1p plays a role critical for sister-chromatid cohesion. The cohesion defect observed for *chl1* mutants is similar in level to those exhibited by other nonessential factors (WANG *et al.* 2000; HANNA *et al.* 2001; MAYER *et al.* 2001). Several findings implicate Chl1p in cohesion establishment, including genetic and physical interactions with Ctf7p and the finding that point mutations in the DNA helicase domain I abrogate Chl1p function in chromosome transmission (HOLLOWAY 2000). Thus, Chl1p may be the first component of the DNA replication machin-

ery to encounter DNA sites destined for cohesion establishment (Skibbens 2000). These findings suggest a stepwise assembly or modification of cohesion sites prior to the emergence of the newly replicated sister chromatids. Previous studies of *chl1* mutant strains resulted in an apparent paradox: Chl1p is a DNA helicase-like protein but defects in Chl1p resulted in a mitotic delay that did not require the DNA damage checkpoint pathway (Gerring *et al.* 1990). Instead, *chl1* mutants were severely growth compromised when combined with a mutation in the kinetochore/spindle checkpoint pathway (Li and Murray 1991). The finding that Chl1p is critical for sister-chromatid cohesion helps resolve this paradox in that defects in cohesion are known to activate the kinetochore/spindle assembly checkpoint and not the DNA damage checkpoint (Skibbens *et al.* 1999). The observation that loss of Chl1p function, when combined with loss of Ctf18p function, is lethal suggests that these two nonessential factors act to establish sister-chromatid cohesion through independent but parallel pathways: namely via DNA helicase activity and through RFC clamp loading functions. Thus, DNA helicases represent a novel pathway that facilitates sister-chromatid cohesion.

Reciprocal database searches reveal that, of the entire budding yeast genome, yeast Chl1p exhibits the highest degree of sequence similarity to human BACH1. Interestingly, while the similarity between human BACH1 and human CHLR1 was noted, alignments were instead shown between BACH1 and yeast Rad3p (Cantor *et al.* 2001). At this point, it remains unknown whether either budding yeast Rad3p or Chl1p is the functional ortholog of either human BACH1 or CHLR1 (Amann *et al.* 1997; Cantor *et al.* 2001). In contrast, recent findings reveal that hCHLR1 exhibits DNA helicase activities and that point mutations in the ATP-binding domain of budding yeast Chl1p abrogate its function in chromosome transmission (Amann *et al.* 1997; Hirota and Lahti 2000; Holloway 2000). Given that budding yeast Chl1p functions in cohesion and is the closest fit to both human BACH1 and CHLR1, it is worth speculating about the role that human DNA helicases may play in cohesion establishment and genome maintenance. For instance, BACH1 helicase directly interacts with the tumor suppressor BRCA1 and plays a key role in BRCA1-dependent repair of double-strand breaks, presumably by unwinding DNA near the site of DNA damage (Kerr and Ashworth 2001; Venkitaraman 2002). Notably, cells harboring BRCA1 pathway mutations exhibit not only translocations and double-strand breaks, but also a variety of aberrant chromosome configurations, including gaps between sister chromatids (Deming *et al.* 2001). It is therefore feasible that the BACH1 helicase associates with BRCA1 not only to repair double-stranded breaks, but also to establish cohesion during DNA repair. On a local scale, loss of sister-chromatid pairing could defeat double-stranded break repair. On a larger scale, global defects in sister-chromatid pairing could significantly contribute to the genetic instability reported in cancer cells associated with BACH1 and BRCA1 loss of function. A growing but circumstantial body of evidence supports a role for a CTF7-like protein in BRCA1-BACH1 genome maintenance. First, human BRCA1 has been found in complexes containing the DNA helicase BACH1 and RFC subunits (Bochar *et al.* 2000; Cantor *et al.* 2001; Deming *et al.* 2001). In budding yeast, Ctf7p physically interacts with Chl1p (a protein that exhibits significant similarity to BACH1) and with all RFC complexes identified to date (Kenna and Skibbens 2003; present study). Second, BRCA1 is recruited to PCNA foci upon DNA damage (Scully *et al.* 1997; Cantor *et al.* 2001). In budding yeast, multiple genetic interactions have been found between *CTF7* and *POL30* (PCNA), indicating that Ctf7p and PCNA functions are intimately coupled (Skibbens *et al.* 1999). Third, BRCA1 is part of a complex containing SWI/SNF chromatin-remodeling factors that exhibit acetyltransferase activity. In budding yeast, Ctf7p is an acetyltransferase, although the targets of Ctf7p-dependent acetylation remain unknown (Ivanov *et al.* 2002).

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