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The budding yeast protein Chl1p has a role in transcriptional silencing, rDNA recombination, and aging

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Abstract

We show that the budding yeast protein Chl1p, required for sister-chromatid cohesion, also modulates transcriptional silencing at *HMR* and telomeres. The absence of this protein results in increased silencing at *HMR* and, conversely, in decreased silencing at the telomere. The regulation of silencing by Chl1p at these two loci is dependent on the presence of Sir proteins. Chl1p also acts synergistically with Sir2p to suppress rDNA recombination. In the absence of this protein, yeast cells exhibit reduced life span and hypersensitivity to heat stress. These observations suggest a role of Chl1p in regulating chromatin structure.

Keywords: Chl1p; Yeast; Transcriptional silencing; Sister-chromatid cohesion; rDNA recombination; Sir proteins; Heat shock; Life span; Aging

In the budding yeast *Saccharomyces cerevisiae*, the cryptic mating type loci (*HMR* and *HML*), regions adjacent to the telomeres and the rDNA array, are associated with silenced domains which are repressive to transcription by RNA polymerase II (reviewed in [1–4]). Sir1–Sir4 (silent information regulators) proteins are the chief architects of a silenced domain and were first identified by mutations in genes that led to the loss of silencing at the mating type loci (reviewed in [5,6]). Sir proteins are recruited to the silencer regulatory sites by protein–protein interactions. Sir2p has NAD^+-dependent histone deacetylase activity and, once recruited to chromatin, mediates transcriptional silencing by deacetylating histone H3 and H4 tails of neighboring nucleosomes (reviewed in [7,8]). Different complexes of Sir proteins effect silencing at different sites. All of Sir proteins are required for silencing at the silent mating type loci [9]. Sir2p, Sir3p, and Sir4p are needed at the telomeric regions [10], although the role of Sir1p in silencing at some native telomeres has been reported [11]. Sir2p is the only Sir protein that appears to be involved at rDNA repeats for silencing [12,13] and in suppressing recombination [14].

The budding yeast protein, Chl1p, was first identified by its role in chromosome maintenance [15,16] and more recently for the establishment of sister-chromatid cohesion [17–19]. It also affects mating type donor selection [20]. Chl1p is a putative helicase, has an essential ATP-binding site, is localized to the nucleus, and has a human homolog BACH1 that binds to the tumor suppressor protein BRCA1 and helps in its repair function [19,16,21,22]. An interplay between sister-chromatid cohesion, chromatin remodeling factors, and transcriptional silencing has been reported in the budding yeast and other organisms [23,24]. We, therefore, investigated if Chl1p was involved in transcriptional silencing at the silent loci in budding yeast.

Materials and methods

Media and chemicals. Rich (YEPD), complete (com) synthetic, dropout synthetic, 5-fluoroorotic acid (5-FOA), and canavanine-containing media have been described before [25,26]. Limiting adenine medium contained 6 μg/ml adenine. Canavanine and 5-FOA were from Sigma, USA.

Strains. All yeast strains used in this study are listed in Table 1. The triple silencer strain CCFY 100 was used to monitor transcriptional

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Table 1
List of strains

Strain	Genotype	Reference/source
CCFY100	W303 *MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11,15 can1-100 hmrΔE::TRP1 rDNA::ADE2 CAN1 VRTEL::URA3*	[33]
sir3Δ	W303 *MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11,15 can1-100 sir3::HIS3*	N. Roy
sir4Δ	W303 *MAT***a** *ade2-1 ura3-1 trp1-1 leu2-3,112 his3-11,15 can1-100 sir4::HIS3*	N. Roy
SL12	W303 *MATα leu2-3,112 his3-11,15 trp1-1 ade2-1 ura3 chl1::HIS3*	This study
SPD1	CCFY100 *chl1::HIS3*	This study
SPD2	CCFY100 *sir1::LEU2*	This study
SPD3	SPD2 *chl1::HIS3*	This study
SPD4	CCFY100 *sir2::LEU2*	This study
SPD5	SPD4 *chl1::HIS3*	This study
SPD6	CCFY100 *sir3::HIS3*	This study
SPD7	SPD6 *chl1::LEU2*	This study
SPD8	CCFY100 *sir4::HIS3*	This study
SPD9	SPD8 *chl1::LEU2*	This study
SPD10	*MATα leu2-3,112 his3-11,15 trp1-1 ade2-1 ura3 hmrΔE::TRP1*	Spores obtained from the cross between SL12 X CCFY100
SPD11	*MATα leu2-3,112 his3-11,15 trp1-1 ade2-1 ura3 hmrΔE::TRP1 chl1::HIS3*	Spores obtained from the cross between SL12 X CCFY100
SPD12	*MATα leu2-3,112 his3-11,15 trp1-1 ade2-1 ura3 VRTEL::URA3*	Spores obtained from the cross between SL12 X CCFY100
SPD13	*MATα leu2-3,112 his3-11,15 trp1-1 ade2-1 ura3 VRTEL::URA3 chl1::HIS3*	Spores obtained from the cross between SL12 X CCFY100
A3	*MAT***a** *leu2-3,112 his3-11,15*	[25]
301-2B	*MATα leu2-3,112 ura3-52 his4Δ34 trp1*	[48]
AP22	*MATα leu2-3,112 his3-11 ura3-52 trp1*	From A3 X 301-2B
AP22Dchl1	*MATα leu2-3,112 his3-11 ura3-52 trp1 chl1::HIS3*	By disrupting *CHL1* in AP22

silencing. The CCFY100 strain contained *TRP1* inserted at the Rap1p-binding site at *HMR-E*, *URA3* inserted at the telomere of chromosome VR, and the *ADE2-CAN1* marker inserted in the rDNA. Gene disruptions and deletions were made as follows. The 3.3 kb *Eco*RI fragment carrying *CHL1* ORF was cloned in the plasmid YIplac211 [27] to get YIplac211-*CHL1*. A 1.7 kb *Bam*HI fragment from pYAC4 [28], carrying the *HIS3* gene, was cloned into the *Bgl*II site of *CHL1* in YIplac211-*CHL1*. The 5 kb *Eco*RI fragment carrying *CHL1* disrupted with *HIS3* was used for transformation to disrupt *CHL1* on the chromosome [29]. For *CHL1* deletions using *LEU2*, the 2.9 kb *Bgl*II fragment carrying *LEU2* from YEp13 [30] was cloned into the *Bgl*II site of YIplac211-*CHL1*. The 6.5 kb *Pvu*II fragment carrying *CHL1* disrupted with *LEU2* was used for transformation. Genomic DNA isolated from sir3Δ and sir4Δ strains deleted with *HIS3* (described in Roy and Runge [31]) was used as template for PCR to delete *SIR3* or *SIR4* genes in present studies. For deleting *SIR3* the primers were 5′ GGGGAACAAAGTATTCGGGACG 3′ (from 261 bp upstream of ATG of *SIR3*) and 5′ CGCGCAGGTGAGAG AGTCCTGG 3′ (287 bp downstream of stop codon). For deleting *SIR4* primers were 5′ CATGTGCACTGCCATTAAG 3′ (267 bp upstream of ATG of *SIR4*) and 5′ GTGGCAAGGTCGGTTTGG 3′ (325 bp downstream of stop codon). The PCR fragments were used for transformation to get *sir3* or *sir4* deletions with *HIS3* as the marker. PCR-mediated deletions using *LEU2* as the marker were according to Longtine et al. [32]. The template used for PCR amplification of *LEU2* was YCp1 [25]. For deleting *SIR1*, the forward hybrid primer was 5′ GCGAGCGAGT CAGCAAGCAGAATCTAAAGAGGCTTGCAACGGACCGCAGTTA ACTGTGGG 3′ of which the first 41 bp are from *SIR1* (234 bp upstream of ATG) and the next 19 bp of *LEU2* (418 bp upstream of ATG). The reverse hybrid primer was 5′CCCGCTTATATGTTGGTATCCA TAACTGATAATCTTACCGAGGAGGTCGACTACGTCG 3′ of which the first 39 bp were from *SIR1* (45 bp downstream of the stop codon) and the next 19 bp from *LEU2* (484 bp downstream of the stop codon). For deleting *SIR2*, the forward hybrid primer was 5′ GACTGGTGCAGGTGTTTCAACTTCATTAGGGATCCCGGACGA CCGCAGTTAACTGTGGG 3′ of which the first 40 bp are from *SIR2* (780 bp downstream from the start codon) and the next 19 bp of *LEU2* (418 bp upstream of ATG). The reverse hybrid primer was 5′ GT TTGCCATACTATGTAAATTGATATTAATTTGGCACGAGGAGGT CGACTACGTCG 3′ of which the first 37 bp were from *SIR2* (63 bp downstream of the stop codon) and the next 19 bp from *LEU2* (484 bp downstream of the stop codon). All the deletions were confirmed by PCR.

Transcriptional silencing assay. This was done exactly as described in Roy and Runge [33]. Log phase cells were streaked on YEPD plates for single colonies. Similar-sized colonies were taken from each strain and resuspended in 500 μl water. Five microliter aliquots of undiluted cells and tenfold serial dilution were spotted in each row on appropriate plates.

Determination of rDNA recombination rates. The loss of an *ADE2* marker integrated into the rDNA array was used to measure recombination. A colony from each strain was grown to mid-log phase in liquid YPD medium, diluted, and plated onto synthetic complete plates with limiting adenine for the development of color. Colonies were allowed to grow for 48 h at 30 °C and then placed at 4 °C for 4 days prior to analysis. Colonies were scored for the red color as described in Merker and Klein [34].

Life span assay. Micromanipulation and life span analysis were performed as described previously (Kennedy et al. [35]). A single colony was inoculated and grown in 5 ml YD and 5 μl was streaked on agar plate. An appropriate number of individual cells were randomly picked under a microscope and aligned in isolated areas with a micromanipulator. Fresh daughters (new buds) were isolated and placed at defined positions away from the mother cell. The number of buds given by this virgin daughter was monitored.

Heat shock assay. A single colony was resuspended in 700 μl water and heated at 55 °C for 30 min. Aliquots were taken out and ten-fold serial dilutions were made and then 5 μl aliquots of cells were spotted on rich medium.

Telomere length determination. Approximately 6 μg of yeast genomic DNA was digested with *Xho*I and fractionated on 0.8% agarose gel. The Southern blot of this DNA was hybridized with poly(d[CA/GT]) probe to detect telomere DNA, as described in [36].

Results and discussion

chl1 affects transcriptional silencing at the HMR locus and telomeres

Transcriptional silencing was analyzed in the wild-type strain CCFY100 ([33], Table 1) and in its isogenic mutant derivative SPD1 (*chl1*). At the mating type locus silencing was monitored from the expression of *TRP1*, at the telomeric region by the expression of *URA3*, and at rDNA by the expression of the *CAN1* gene inserted at this locus. These loci show incomplete silencing in the wild type, so that increase or decrease in silencing could be monitored. Fig. 1A shows that in the *chl1* mutant the expression of *TRP1* was reduced while that of *URA3* was increased, suggesting that Chl1p antagonizes the formation of repressed chromatin at *hmrΔE::TRP1* while it favors transcriptional repression at telomeres. No change was observed in the growth of mutant and wild-type cells on canavanine plates (not shown), suggesting that *chl1* does not affect silencing at rDNA. *chl1 MAT*α cells behaved similarly to *chl1 MAT***a** cells; silencing was increased at the *hmrΔE* locus and decreased at the telomere (Fig. 1B). High copies of Chl1p did not suppress these phenotypes (Fig. 1C), indicating that normal cellular levels of Chl1p are not limiting for its silencing-related function. Several mutants which affect transcriptional silencing, such as *sir3*, *sir4*, *rad53*, and *mec1*, also affect the lengths of telomeres [37,38]. We, therefore, investigated the role of Chl1p in the maintenance of telomere length. No change was observed in telomere length in the *chl1* mutant, nor any synergistic effects were obtained when *chl1* was combined with *sir3* or *sir4* (Fig. 1D). This suggests that Chl1p does not affect telomere length.

Chl1p acts in the SIR-dependent pathway of silencing

To test if Chl1p was influencing the SIR-dependent pathway of transcriptional silencing at *HMR* and telomeres, the double mutants *sir1 chl1*, *sir2 chl1*, *sir3 chl1*, and *sir4 chl1* were spotted for growth on −Trp and on FOA plates. All the double mutants lost silencing at *hmrΔE* like their *sir* parents, as seen from their growth on −Trp plates (Fig. 2), suggesting that increase in silencing at *HMR* observed in the *chl1* mutant needed Sir proteins. Thus, Chl1p, directly or indirectly, interferes with the function of Sir proteins in establishing silencing at this locus. Apart from *sir1 chl1*, all other double mutants, like their *sir* parents, had derepressed chromatin at the telomere (Fig. 2), suggesting that Chl1p acts through Sir proteins to silence chromatin at telomeres. Sir1p does not affect silencing associated with the telomere VR construct used in these strains [10]. This was consistent with our observations that *sir1* mutant, like the wild type, still showed repressed chromatin at this telomere and that *sir1 chl1* was less repressed than *sir1*. This result also shows that the loss of silencing at this telomere in *chl1* was not due to a titration of Sir2, Sir3 or Sir4 proteins from telomeres to *hmrΔE*.

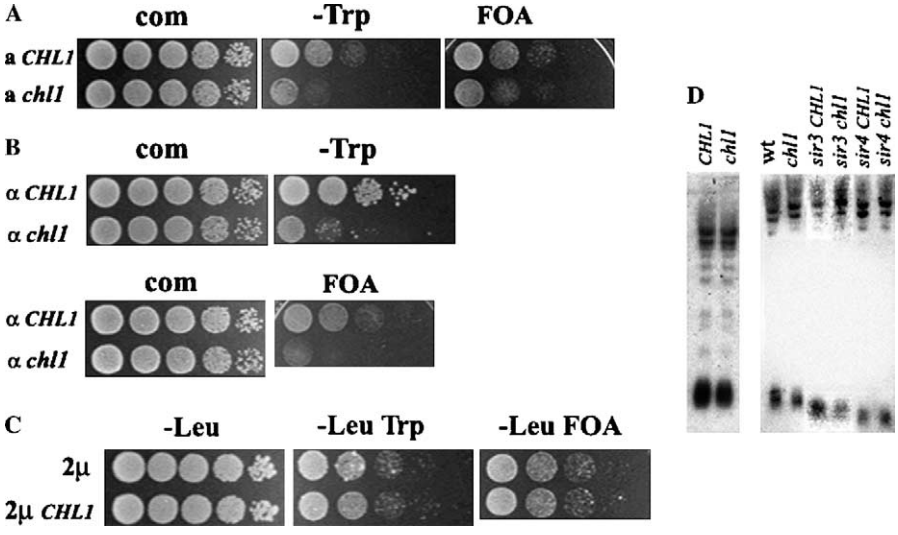

Fig. 1. Deletion of *CHL1* causes changes in transcriptional silencing. To monitor silencing at *hmrΔE::TRP1* and *VRTEL::URA3*, cells were serially diluted and spotted on the indicated media as described in Roy and Runge [33]. On FOA plates, only first four dilutions are shown. (A) Growth of CCFY100 (*CHL1*) and the isogenic mutant SPD1 (*chl1*) strains on complete synthetic medium (com), complete synthetic medium lacking tryptophan (−Trp), and on FOA medium (B) *chl1* also affects silencing in *MAT*α cells. Growth of SPD10 (*MAT*α *CHL1 hmrΔE::TRP1*) and SPD11 (*MAT*α *chl1::HIS3 hmrΔE::TRP1*) on complete and −Trp media while that of SPD12 (*MAT*α *CHL1 VRTEL::URA3*) and SPD13 (*MAT*α *chl1::HIS3 VRTEL::URA3*) on complete and FOA media. (C) High copies of Chl1p do not influence silencing at the *hmrΔE::TRP1*, nor at the telomere VR in the wild type. Growth of CCFY100 (*CHL1*) transformed with the 2μ-based plasmid YEplac181 [27] and with YEplac181-*CHL1* where the *CHL1* ORF was present in a 3.3 kb *Eco*RI genomic fragment that complements the *chl1* mutation in a single copy plasmid [29]. (D) Absence of Chl1p does not affect telomere length. The Southern blot of *Xho*I-digested genomic DNA was hybridized with poly(d[CA/GT]) probe to detect telomeric DNA [36]. Strains used were wild type (CCFY100), *chl1* (SPD1), *sir3* (SPD6), *sir3 chl1* (SPD7), *sir4* (SPD8), and *sir4 chl1* (SPD9).

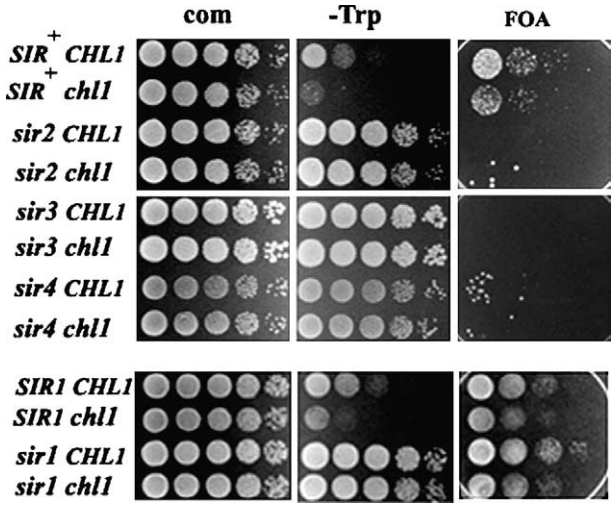

Fig. 2. Chl1p needs SIR proteins for its silencing function. Strains used were *SIR*$^{+}$ *CHL1* (wild type), *chl1* (SPD1), *sir1* (SPD2), *sir1 chl1* (SPD3), *sir2* (SPD4), *sir2 chl1* (SPD5), *sir3* (SPD6), *sir3 chl1* (SPD7), *sir4* (SPD8), and *sir4 chl1* (SPD9). Experimental details were as in Fig 1.

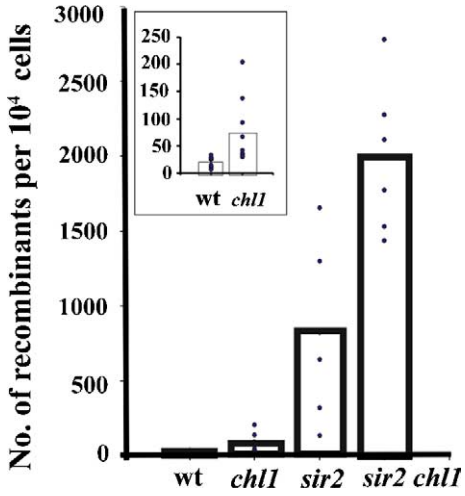

Fig. 3. Chl1p works synergistically with Sir2p to affect unequal sister-chromatid recombination rate at rDNA repeats. Recombination was expressed as the number of recombinants (red-sectored colonies) per 10^4 cells. The recombination frequency determined from each experiment is represented by a dot, while the average recombination frequency is represented by a bar. The number of independent experiments was: nine each for wild type (CCFY100) and *chl1* (SPD1), six each for *sir2* (SPD4), and *sir2 chl1* (SPD5).

In such a case, these proteins should have been available in the *sir1 chl1* mutant (due to the disruption of silencing at *hmrΔE*) to re-establish wild-type silencing at the telomere.

chl1 mutation increases the rate of unequal sister-chromatid recombination within rDNA

In the absence of Sir2p, there is a decrease in cohesin association at the rDNA repeats, leading to an increase in unequal sister-chromatid recombination rate and loss of a marker gene inserted at this site [39]. Since Chl1p is also required for the establishment of sister-chromatid cohesion, we checked if the absence of this protein increased unequal sister-chromatid recombination at rDNA, leading to the excision of the *ADE2* gene inserted at this locus in the strains used. Loss of *ADE2* in a cell of a growing colony would make it red-sectored, and the frequency of appearance of such colonies would reflect the unequal sister-chromatid recombination rate. A colony from each strain was grown to mid-log phase in liquid YEPD medium, diluted, and plated onto synthetic complete plates with limiting adenine (6 μg/ml) for the development of color as described in Merker and Klein [34]. The rate of recombination was found to be about fourfold higher in the mutant than in the wild-type strain (Fig. 3, inset). Therefore, Chl1p is required to suppress unequal sister-chromatid exchange at the rDNA locus. To see if Chl1p acted in the Sir2p pathway to suppress recombination rate, the double mutant *sir2 chl1* was also analyzed in this experiment. Recombination occurred with a much higher frequency in the double mutant than in either of the parents (Fig. 3), suggestive of synergistic interactions between the two mutations. Therefore, it is reasonable to presume that Chl1p and Sir2p have overlapping functions in suppressing unequal sister-chromatid recombination at rDNA by regulating sister-chromatid cohesion.

Chl1p affects aging and resistance to heat stress in yeast

Increased recombination at the rDNA locus is generally associated with loss in longevity due to the accumulation of extrachromosomal rDNA circles (ERCs) [40–42]. For this reason, the life span of the *chl1* null mutant was compared with that of the wild type. Fig. 4A shows that, on an average, the mutant cells divided for fewer generations as compared to the wild-type cells. It has been shown that aging cells tend to accumulate oxidatively damaged proteins [43], which suggests the accumulation of ROS (reactive oxygen species) and explains the inability of these cells to cope efficiently with stress [44]. Heat stress leads to oxidative stress and several genes induced by heat shock are also induced by oxidative stress [45]. Since *chl1* cells age early, we compared the tolerance of wild-type and mutant cells towards heat stress. *chl1* cells were more susceptible to heat stress (Fig. 4B), consistent with the observation that enhanced stress resistance is associated with longer life span [44].

In summary, we have shown that Chl1p is involved in the maintenance of silencing at the *HMR* and telomeres and acts through the Sir-dependent pathway. This protein is also needed to prevent unequal sister-chromatid exchange at rDNA where it acts synergistically with Sir2p. Finally, Chl1p is required to prevent premature aging of cells and in stress tolerance. Although Chl1p is dependent on Sir proteins for regulating silencing at *HMR* and telomere, no physical interaction between Chl1p and Sir proteins (or with Rap1p) was uncovered by two-hybrid studies in this present work (data not shown). Therefore, Chl1p may not directly interact with these proteins to regulate silencing at *HMR* or telomeres. Instead, defects in

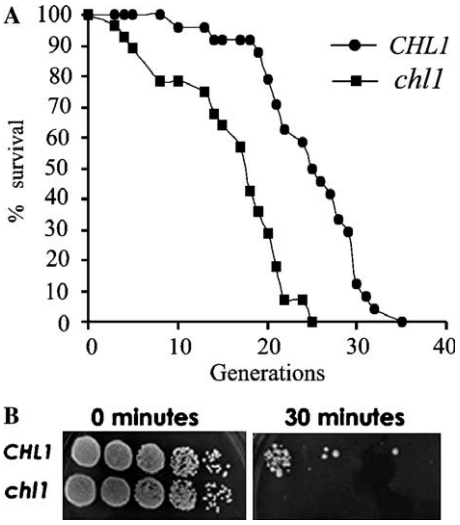

Fig. 4. Chl1p is required for longevity and for resistance towards heat shock. (A) Survival curves for the wild-type (AP22) and mutant (AP22Dchl1) showing the percentage of cells surviving after a given number of divisions. The mean and maximum life spans were 25 and 35 divisions for the wild-type cells and 16 and 25 for the mutant. The curves represent samples of 24 cells for the wild-type and 28 cells for the mutant. Micromanipulation was done as described in Kennedy et al. [35]. (B) *chl1* causes increased sensitivity towards heat shock. Survival of the wild-type (AP22) and mutant (AP22Dchl1) cells exposed to 55 °C heat shock for 30 min, as described in Roy and Runge [33].

cohesion establishment during replication could be altering chromatin structure with consequent alterations in transcriptional silencing. This needs to be verified by further experiments.

In the budding yeast, cohesion is reported to play both positive and negative roles in transcriptional silencing. While cohesion establishment proteins Ctf4, Ctf18, and Dcc1 are all required for transcriptional silencing at *HMR* and telomeres [23], cell cycle studies show that sister-chromatid cohesion needs to be destroyed for the establishment of proper silencing at *HMR* in late M-phase [46]. Mutation of another cohesin subunit, *SMC1*, causes a loss of boundary function at *HMR* and results in a further spread of silencing [47]. Chl1p plays a negative role at *HMR* and a positive one at the telomeres. The differences in the phenotypes of *chl1* and *ctf4*, etc., could reflect some fundamental differences in the mechanisms by which the corresponding proteins establish cohesion and affect chromatin structure. Although the detailed molecular mechanisms by which Chl1p acts are being worked out, the present observations suggest that Chl1p plays a crucial role in regulating chromatin structure in yeast and links sister-chromatid cohesion with transcriptional silencing. Furthermore, our studies should help throw light on the function of BACH1, the human homolog of Chl1p.

Acknowledgments

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