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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].