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The Yeast GAL11 Protein Is Involved in Regulation of the Structure and the Position Effect of Telomeres

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GAL11 is an auxiliary transcription factor that functions either positively or negatively, depending on the structure of the target promoters and the combination of DNA-bound activators. In this report, we demonstrate that a *gal11*Δ mutation caused a decrease in the length of the telomere $C_{1-3}A$ tract, a derepression of *URA3* when it is placed next to telomere, and an increase in accessibility of the telomeric region to *dam* methylase, indicating that *GAL11* is involved in the regulation of the structure and the position effect of telomeres. The defective position effect in a *gal11*Δ strain was suppressed by overproduction of SIR3, whereas overexpression of *GAL11* failed to restore the telomere position effect in a *sir3*Δ strain. Hyperproduced GAL11 could partially suppress the defect in silencing at *HMR* in a *sir1*Δ mutant but not that in a *sir3*Δ mutant, suggesting that *GAL11* can replace *SIR1* function partly in the silencing of *HMR*. Overproduced SIR3 also could restore silencing at *HMR* in *sir1*Δ cells. In contrast, *SIR1* in a multicopy plasmid relieved the telomere position effect, especially in a *gal11*Δ mutant. Since chromatin structure is thought to play a major role in the silencing at both the *HM* loci and telomeres, *GAL11* is likely to participate in the regional regulation of transcription by modulating the chromatin structure.

Chromatin is known to play a role in transcriptional regulation (reference 15 and references therein). When chromatin structure is studied by DNase I digestion, active genes in the yeast *Saccharomyces cerevisiae*, like those in higher eukaryotes, display enhanced sensitivity to DNase I at sites neighboring the active genes. Using *Escherichia coli dam* methylase expressed in yeast cells, Singh and Klar have shown that transcriptionally active genes are methylated more efficiently in their promoter and coding regions than are inactive genes, indicating that active genes (chromatin) have an increased accessibility to this enzyme as well (46). Studies of *S. cerevisiae* have provided both biochemical and genetic evidence that nucleosomes, the primary components of chromatin, affect transcription: derepression of the *PHO5* gene under low-phosphate conditions removes two nucleosomes, positioned both upstream and downstream of an upstream activating sequence of the gene (9). *SNF2* (identical to *SWI2*) and *SNF5* cause alteration in chromatin structure in the *SUC2* promoter, leading to transcriptional activation (17). *SIN4* (*TSF3*) exerts positive or negative effects on transcriptional activation of different genes, and mutations in *SIN4* alter the superhelical density of plasmids which stem from changes in chromatin structure (6, 20).

S. cerevisiae has two major transcriptionally inactive loci, the *HM* silent mating-type loci (2) and the telomere regions (14), both of which exert a position effect on the transcription of neighboring genes. The silencers flanking both *HML* and *HMR* loci are composed of multiple elements, including an autonomously replicating sequence and RAP1- and ABF1-binding sites (26). In addition, *SIR1*, *SIR2*, *SIR3*, *SIR4*, *NAT1*, *ARD1*, and *HHF1*/*HHF2* are required in *trans* for *HM* silencing (19, 22, 30, 38, 53). A deletion removing amino acids 4 to 19 from histone H4 results in loss of silencing at both *HML* and *HMR*, indicating involvement of chromatin structure in *HM* silencing (22). *SIR1* and *SIR3* were shown to interact genetically with histone H4 in silencing (21, 47). Additional evidence for the involvement of chromatin in silencing was provided by nuclease sensitivity analysis of *HML* and *HMR*, showing that the *HM* loci assume a distinct chromatin structure that is dependent on the *SIR* genes (31). Gottschling et al. (14) demonstrated that genes placed adjacent to the telomere $C_{1-3}A$ repeat are transcriptionally repressed and that the seven genes involved in the *HM* silencing, except *SIR1*, are also required for the telomere position effect (1). From these results, they suggested that telomeres, like the *HM* loci in *S. cerevisiae*, exist in a distinct chromatin structure. In accord with this hypothesis, yeast telomeres were shown to assume a nonnucleosomal chromatin structure (54).

The *GAL11* gene can exert either a positive or negative effect on gene expression (10, 16, 35, 50), and we have found that the effects of *GAL11* depend on the structure of promoter and a combination of DNA-bound factors (35a). In a study of the *GAL11* effect on expression of the *SUC* genes, we found that *GAL11* regulates *SUC2* differently from *SUC7*, which lies proximal to a telomere. *GAL11* is required for efficient transcriptional activation of *SUC2* (51); expression of both *SUC2* and *SUC7* is regulated by glucose, and their promoter sequences are conserved in the regions containing the TATA box and the upstream regulatory sequences (41). However, their chromosomal locations are different; *SUC7* is located very close to telomere (4 kbp away from the Y′ element), whereas *SUC2* is at least 14 kbp from telomere-adjacent sequences (4). In fact, *SUC2* is an exceptional member of the *SUC* gene family (*SUC1* through *SUC5* and *SUC7*), the other members of which are located very close to telomeres (4). This raises a possibility that *SUC7* is under the telomere position effect and that *GAL11* affects *SUC7* expression by altering the telomere position effect. *GAL11* is required for transcriptional activation of *MAT*α and *PYK1* by the RAP1 protein (11, 23, 33, 35), and RAP1 binds to the telomere $C_{1-3}A$ repeat and is involved in regulation of both telomere length and the position effect (3, 8, 24, 25, 28, 45, 48). These considerations prompted us to

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TABLE 1. Genotypes and sources of yeast strains used

Strain	Genotype	Source
HSY5-3C	*MATα ade1 his1 leu2 trp1 ura3-52 SUC2 SUC7*	Our laboratory (35a)
HSY5-3B	HSY5-3C *gal11Δ::LEU2*	Our laboratory (35a)
MCY1094	*MATα ade2-101 ura3-52 SUC2*	M. Carlson
YS60	MCY1094 *gal11Δ::URA3*	This work
MCY526	*MATα his4-539 lys2-801 ura3-52 SUC7 GAL*$^+$	M. Carlson
YS26	MCY526 *gal11Δ::URA3*	This work
MCY517	*MAT***a** *his4-539 lys2-801 ura3-52 SUC2 SUC7 GAL*$^+$	M. Carlson
YS17	MCY517 *gal11Δ::URA3*	This work
YS38	HSY5-3C *sir1Δ::LEU2*	This work
YS39	HSY5-3C *sir3Δ::LEU2*	This work
YS40	HSY5-3C *sir4Δ::LEU2*	This work
YS41	YS38 *hmra::TRP1*	This work
YS43	YS39 *hmra::TRP1*	This work
YS44	YS40 *hmra::TRP1*	This work
YS92	HSY5-3C *URA3*$^+$	This work
YS99	HSY5-3C *URA3*$^+$ *gal11Δ::LEU2*	This work
YS93	HSY5-3C *gal11Δ::URA3*	This work
YS94	HSY5-3C *adh4*::TEL-*URA3*	This work
YS95	HSY5-3C *gal11Δ::LEU2 adh4*::TEL-*URA3*	This work
YS97	HSY5-3C *sir3Δ::LEU2 adh4*::TEL-*URA3*	This work
YS98	HSY5-3C *sir4Δ::LEU2 adh4*::TEL-*URA3*	This work

examine whether *GAL11* is involved in the regulation of the structure and function of telomeres. The results presented in this report demonstrate that *GAL11* is required for maintenance of the normal length of the telomere $C_{1-3}A$ repeat and for the telomere position effect and that *GAL11* alters chromatin structure near the telomere. We further show a possible participation of *GAL11* in *HMR* silencing. From these results, we present a model for the mechanisms by which *GAL11* regulates transcription of genes at telomeres as well as at the promoters.

MATERIALS AND METHODS

Media and strains. *E. coli* HB101 and JM109 were used to construct plasmids and were grown in Luria-Bertani medium supplemented with ampicillin (80 μg/ml) when necessary. *S. cerevisiae* strains used in this study are listed in Table 1. YS38, YS39, and YS40 were constructed by using plasmids pJH570, containing the *SIR1* gene, pJH107.1, containing *sir3::LEU2* disruption, and pKAN59, containing the *SIR4* gene, obtained from J. M. Ivy via S. Harashima (19). Plasmid pJH570 was cut with *Xho*I and *Bgl*II, and the 1.1-kbp fragment was replaced with a fragment with corresponding ends carrying a *LEU2* marker. The resulting plasmid was cut with *Pst*I and then used to transform *S. cerevisiae* HSY5-3C cells. Plasmid pJH107.1 was cut with *Bam*HI before being used to transform HSY5-3C cells. Plasmid pKAN59 was digested with *Pvu*II to create a gap that was filled with a DNA fragment containing the *LEU2* marker. The resulting plasmid was cut with *Sph*I and then used to transform *S. cerevisiae* HSY5-3C cells. Disruption of the *HMR* locus in strains YS38, YS39, and YS40 was carried out essentially as described previously (39, 48): an *Xba*I-*Bgl*II fragment containing X and Ya regions was replaced by an *Eco*RI-*Bgl*II fragment containing *TRP1* to generate strains YS41, YS43, and YS44, respectively. Strains YS17, YS26, YS60, and YS93 were constructed by replacing a 2.5-kbp *Sac*I-*Eco*RV fragment containing the coding region of *GAL11* (50) in MCY517, MCY526, MCY1094, and HSY5-3C, respectively, with a *URA3* marker carried on a 1.1-kbp *Hin*dIII fragment. Strain YS92 was constructed by replacing the *ura3-52* locus with the wild-type *URA3* gene carried on a 1.1-kbp *Hin*dIII fragment. Strain YS99 was then constructed by disrupting the *GAL11* locus of YS92 through replacement of a 2.5-kbp *Sac*I-*Eco*RV fragment containing the coding region of *GAL11* (50) with a DNA fragment containing the *LEU2* marker. Strains YS94, YS95, YS97, and YS98 were constructed by introducing *URA3* into the terminus of the left arm of chromosome VII by using plasmid pVII-L *URA3*-TEL (a gift of D. E. Gottschling) (14) in strains HSY5-3C, HSY5-3B, YS39, and YS40, respectively.

Construction of plasmids. DNA manipulations and genetic techniques, including procedures for *E. coli* and yeast transformations, were carried out as described previously (40, 44). A YCp-based plasmid containing the *E. coli dam* methylase gene carried on a 1.5-kbp *Hin*dIII-*Pvu*II fragment from plasmid pMFH1 (a gift of R. E. Malone) (18) was constructed by replacing the *Xho*I-*Bam*HI fragment of plasmid pMF527C (35a). Plasmid pYS81, containing the full-length *GAL11* and *dam* genes, was constructed by introducing the 1.5-kbp *Hin*dIII-*Pvu*II fragment carrying the *dam* gene into an *Xho*I site of plasmid pMF761, a YCp plasmid containing full-length *GAL11* (35a). Plasmid pYS83, containing both the *SIR3* gene and the *dam* gene in a YCp vector, was constructed by introducing a 4.5-kbp *Bam*HI-*Sal*I fragment with the *SIR3* gene from pKAN63 (19) into plasmid pMF527C (35a) previously cut with *Bam*HI and *Xho*I. To the *Xho*I site of the resulting plasmid, the fragment encoding the *dam* methylase with ends converted to *Xho*I sites was inserted. Plasmids carrying the *SIR3* gene in a *TRP1*-marked (pYS43) and a *URA3*-marked (pYS86) YEp vector were constructed, respectively, by inserting a 6.8-kbp *Bam*HI fragment of pKAN63 (19) into plasmid pMF527 (35a) previously cut with *Xho*I and *Bam*HI and into a *Bam*HI site of YEp24. Plasmid pMF858 containing the *SIR1* gene in a *TRP1*-marked YEp plasmid was constructed by replacing the *URA3* marker in plasmid pES13B (a gift of R. Sternglanz) (47) with *TRP1* carried on an 850-bp *Eco*RI-*Bgl*II fragment. Plasmid pMF859 carrying the *SIR4* gene in a *URA3*-marked YEp vector was constructed by introducing a ca. 5-kbp *Eco*RI-*Sal*I *SIR4* fragment from pKAN59 into YEp24.

Plasmids pSUZ1 and pSUZ2 contain the *lacZ* gene driven by the *SUC2* and *SUC7* promoters, respectively, in a YEp vector with a *TRP1* marker. pSUZ1 was constructed by inserting a 1.1-kbp *Eco*RI-*Bam*HI fragment encoding the *SUC2* promoter and the signal peptide of invertase from plasmid pSMF3 (34) into plasmid pMC1403 (5) previously cut with the corresponding enzymes, followed by insertion of a 2.8-kbp *Eco*RI fragment containing a 2μm replication origin and a *TRP1* marker. Plasmid pSUZ2 was constructed by replacing the *Eco*RI-*Bam*HI fragment of pSUZ1 with a fragment composed of a 740-bp *Xba*I-*Hin*dIII fragment from pRT85-3 (a gift of M. Carlson) (41), a 37-bp *Hin*dIII fragment of pRT62-30 (a gift of M. Carlson) (41), and a 70-bp *Hin*dIII-*Bam*HI fragment from pSMF3 (34).

Enzyme and protein assays. Invertase and β-galactosidase were assayed as described previously (12, 33). To repress or derepress the *SUC2* and *SUC7* promoters, cells harboring the appropriate reporter were grown overnight in synthetic complete medium containing 5% glucose and lacking tryptophan (Trp; to maintain *TRP*$^+$ plasmids), harvested, washed twice with water, and resuspended in fresh synthetic complete

TABLE 2. *GAL11* and invertase production

Strain	Genotype[a]	Invertase activity[b] (μmol of glucose/min/ml/A_{600})	
		High glucose	Low glucose
HSY5-3C	*GAL11 SUC2 SUC7*	450	3,220
HSY5-3B	*gal11Δ SUC2 SUC7*	2,150	4,320
MCY1094	*GAL11 SUC2*	130	4,115
YS60	*gal11Δ SUC2*	155	782
MCY526	*GAL11 SUC7*	85	1,155
YS26	*gal11Δ SUC7*	140	1,483
MCY517	*GAL11 SUC2 SUC7*	90	3,195
YS17	*gal11Δ SUC2 SUC7*	280	2,265

[a] Only the relevant genotypes of each strain are shown. *SUC* genes other than those shown are not present. For the complete genotypes, see Table 1.

[b] Values are averages of at least four independent experiments. Standard errors were <20%. High and low glucose designate 5 and 0.1% glucose, respectively. For details of the experiment, see Materials and Methods.

medium containing either 5 or 0.1% glucose. After incubating at 30°C for 3 h, cells were harvested, washed twice with water, and subjected to enzyme assays. Proteins were assayed by Bradford method, using immunoglobulin G as the standard.

Telomere position effect. Expression of telomeric *URA3* (TEL-*URA3*) was determined as resistance to 5-fluoroorotic acid (5-FOA) as described by Gottschling et al. (14). Cells were grown in synthetic complete medium overnight, and serial dilutions were plated onto synthetic complete medium or medium containing 0.04% 5-FOA. The numbers of colonies on a plate were counted after incubation at 30°C for 3 days. 5-FOA resistance was determined by at least four independent experiments and is expressed as the average ratio of colonies formed on synthetic complete medium containing 5-FOA to those formed on synthetic complete medium. For strains harboring a TRP^+ plasmid, colonies formed on synthetic complete medium lacking Trp were suspended in water, and 10-fold serial dilutions were plated onto complete medium either lacking Trp or containing 5-FOA but lacking Trp.

Measurement of the telomere $C_{1-3}A$ repeat length. To measure the length of the telomere $C_{1-3}A$ repeat, total DNA was isolated from $GAL11^+$, *gal11Δ*, or appropriate mutant cells as described previously (44) and digested with *Xho*I, which cuts the DNA at a site within the Y′ telomere element (28). The digested DNA was separated on a 0.8% agarose gel and subject to Southern analysis using a TG_{1-3} 41-mer oligonucleotide (a gift of A. Lustig) (28) labeled with digoxigenin (Boehringer Mannheim) as a probe.

Analysis of *dam* methylation sites. Total yeast DNA was isolated from yeast cells harboring the *dam* gene in a YCp plasmid as described previously (44) and was cut with *Bam*HI and *Hin*dIII (13) and/or other restriction enzymes. Following separation by gel electrophoresis on 0.7% agarose, the fragments were subjected to Southern blot hybridization performed as described previously (40), using a 780-bp *Eco*RV-*Sma*I *URA3* fragment as a probe (13).

RESULTS

A *gal11Δ* mutation reduces the expression of *SUC2* but not of *SUC7*. During work on the effect of *GAL11* on the regulation of *SUC* genes, we found that a *gal11Δ* mutant strain, HSY5-3B, derepressed invertase production under high-glucose conditions and produced an even higher level of the enzyme than the isogenic $GAL11^+$ strain (HSY5-3C) did under low-glucose conditions (Table 2). This result is not consistent with the previous report by Vallier and Carlson that *GAL11* is required for *SUC2* expression (51). Since this strain has both *SUC2* and *SUC7* (data not shown), we next analyzed *GAL11* effect on invertase production in strains that have either *SUC2* or *SUC7*. As shown in Table 2, a *gal11Δ* mutation reduces invertase production by cells having *SUC2* alone (YS60), which confirms the requirement of *GAL11* for full activation of *SUC2* (51). In contrast, a *gal11Δ* $SUC7^+$ strain (YS26) produced invertase at an even higher level than an isogenic $GAL11^+$ $SUC7^+$ strain (MCY526) did under both high- and low-glucose conditions (Table 2). When both *SUC2* and *SUC7* are present, *gal11Δ* mutant cells (YS17) increase invertase production threefold compared with wild-type cells (MCY517) under high-glucose conditions (Table 2).

Taken together, these results suggest that *SUC2* and *SUC7* are differently regulated by *GAL11*. The promoter sequences of the two *SUC* genes, including the upstream regulatory sequences, are highly homologous, which implies that they should be similarly regulated (41, 42). However, *SUC2* and *SUC7* are distinct in their chromosomal locations: *SUC7* is very close to a telomere (4 kbp away from the Y′ element), whereas *SUC2* is not (4). We assume that the difference in chromosomal locations of the two genes is responsible for the difference in regulation by *GAL11*. To test this idea, we assayed the promoter activities of *SUC2* and *SUC7* in plasmids pSUZ1 and pSUZ2 (Table 3). The activities of β-galactosidase expressed under control of the *SUC2* and *SUC7* promoters in plasmids pSUZ1 and pSUZ2, respectively, were reduced by a *gal11Δ* mutation (HSY5-3B; Table 3), indicating that *SUC7* requires *GAL11* for full transcriptional activation when in a plasmid. This finding suggests the possibility that expression of *SUC7* is position dependent.

Transcription of genes located next to telomeres is repressed; this is known as the telomere position effect (14). We inferred that *SUC7* is under the telomere position effect and that a *gal11Δ* mutation might affect *SUC7* expression by altering the position effect. Therefore, we started to examine whether *GAL11* is involved in the position effect at telomeres.

***GAL11* is required to maintain the normal length of the telomere $C_{1-3}A$ tract.** The termini of chromosomes are specified by $C_{1-3}A$ repeat sequences that are normally 250 to 350 bp in length (43, 52). There must be a close relation between the structural integrity of telomeres and the telomere position effect; therefore, we first investigated whether a *gal11Δ* mutation causes an alteration in the length of the $C_{1-3}A$ repeat by Southern analysis of *Xho*I-digested genomic DNA isolated from isogenic $GAL11^+$ or *gal11Δ* cells. As shown in Fig. 1, the telomeric $C_{1-3}A$ repeat became shorter by approximately 120 bp in *gal11Δ* mutant cells (lanes 3 and 4 compared with lanes 1 and 2). This alteration was corrected by introducing a plasmid expressing wild-type GAL11 in a YCp plasmid (lane 6)

TABLE 3. *GAL11* and the promoter activities of *SUC2* and *SUC7*

Reporter plasmid	*GAL11* locus[a]	β-Galactosidase activity[b] (U/mg of protein)	
		High glucose	Low glucose
pSUZ1 (*SUC2-lacZ*)	$GAL11^+$	3	170
	gal11Δ	4	3
pSUZ2 (*SUC7-lacZ*)	$GAL11^+$	9	69
	gal11Δ	17	25

[a] Strains used were HSY5-3C ($GAL11^+$) and HSY5-3B (*gal11Δ*).

[b] Values are averages of at least six transformants. Standard errors were <15%. For other details, see Table 2, footnote *b*.

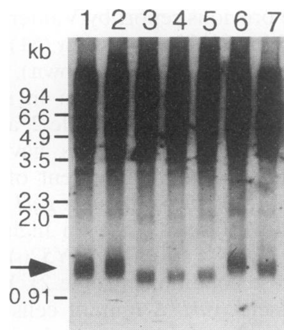

FIG. 1. Effect of a *gal11*Δ mutation on the length of the telomere $C_{1-3}A$ tract. Chromosomal DNA was cleaved with *Xho*I, separated on a 0.8% agarose gel, and analyzed by Southern blotting, using the TG_{1-3} 41-mer (28) as a probe. An arrow indicates the position of the $C_{1-3}A$ tract. Lanes contain DNA from two different isolates of HSY5-3C (*GAL11*$^+$; lanes 1 and 2) or HSY5-3B (*gal11*Δ; lanes 3 and 4) cells harboring the vector, from HSY5-3B (*gal11*Δ) cells harboring *gal11* lacking codons 866 to 910 in a YEp plasmid (lane 5), and from HSY5-3B (*gal11*Δ) cells harboring *GAL11* in a YCp (lane 6) or YEp (lane 7) plasmid. Positions of molecular size markers are shown on the left.

but not by a plasmid expressing a GAL11 derivative lacking the 866 to 910 region (lane 5) that is required for transcriptional activation of *GAL11*-dependent promoters (35a). We also analyzed the length of the telomeric $C_{1-3}A$ repeat in *gal11*Δ mutant cells with a different genetic background (YS11 [50]) and found that it is similarly reduced by the mutation (data not shown). These results indicate that functional *GAL11* is required for the maintenance of normal telomere structure and that the domain of GAL11 essential for this function is the same as that required for transcriptional activation of *GAL11*-dependent genes. It should be noted that overexpression of *GAL11* in a YEp plasmid failed to complement fully the shortening of the $C_{1-3}A$ repeat (lane 7). Hyperproduced GAL11 showed a similar defect in complementation of transcriptional activation of *GAL7* and *PYK1* promoters (35a). We think that an excess amount of the GAL11 protein is inhibitory to its proper function in some cases.

***GAL11* is required for the telomere position effect.** Gottschling et al. reported that expression of *URA3* placed at the terminus of chromosome was repressed, as judged by frequency of 5-FOA-resistant colonies (14), and that *SIR3*, *SIR4*, *ARD1*, *NAT1*, and *HHF* genes are required for this position effect (1). We therefore examined whether *GAL11* plays a role in the telomere position effect by measuring numbers of 5-FOA-resistant colonies of *GAL11*$^+$ or *gal11*Δ strains harboring *URA3* at the terminus of chromosome VII. As shown in Table 4, when *URA3* is at its original locus, both *GAL11*$^+$ and *gal11*Δ cells (YS92 and YS99) were sensitive to 5-FOA. Since the *URA3* locus is far away from the terminus of chromosome V (37), expression of *URA3* at this locus is free from the telomere position effect. While *GAL11*$^+$ TEL-*URA3* cells showed resistance to 5-FOA, the isogenic *gal11*Δ TEL-*URA3* cells showed about a 1,600-fold decrease in the number of 5-FOA-resistant colonies, indicating that expression of TEL-*URA3* was derepressed in the absence of *GAL11*; that is, the telomere position effect was relieved by a *gal11*Δ mutation. Himmelfarb et al. (16) reported that *GAL11* is required for full transcriptional activation of *URA3*. The fivefold increase in the number of 5-FOA-resistant colonies of strain YS99 relative to that of strain YS92 may reflect this. If it were also the case for telomeric *URA3* expression, we should have observed an increase in 5-FOA-resistant cells resulting from a *gal11*Δ mutation. However, the fact that we obtained the opposite result suggests that *GAL11* is dispensable for transcriptional activation of TEL-*URA3* when the telomere position effect is impeded.

***GAL11* and the accessibility of *dam* methylase to telomeres.** The terminal region of the yeast chromosome is proposed to assume a specific chromatin structure responsible for the telomere position effect (1, 14). Gottschling reported that mutations in *SIR3*, *SIR4*, and *HHF2*, genes required for the telomere position effect, altered the accessibility of *E. coli dam* methylase to TEL-*URA3*: a susceptible site was more often methylated in the respective mutant cells, demonstrating that the chromatin structure can be altered as a consequence of any of these mutations (13). Therefore, we also examined whether a *gal11*Δ mutation alters the accessibility of the GATC site in TEL-*URA3* to *dam* methylase. To evaluate the extent of methylation at this site, total DNA from *GAL11*$^+$ and *gal11*Δ cells was cleaved with either *Dpn*I or *Mbo*I, and the efficiencies of cleavage were compared. *Dpn*I cleaves DNA at GATC methylated in both strands, whereas *Mbo*I is unable to cut when either strand is methylated; *Sau*3AI can cut DNA regardless of the methylation state. The results are shown in Fig. 2. In the *gal11*Δ TEL-*URA3* strain (YS95; Fig. 2B, lane 3), the GATC site within TEL-*URA3* was cleaved with *Dpn*I more efficiently than it was in the *GAL11*$^+$ counterpart (YS94; lane 2; compare fragments C and D in lanes 2 and 3). The opposite was observed with *Mbo*I cleavage. *Sau*3AI could cleave the GATC sites completely. Thus, a *gal11*Δ mutation altered the accessibility of the *dam* methylase to TEL-*URA3*, indicating that the chromatin structure near the chromosome terminus was modulated by the mutation. Fragments C and D were not detected in YS93 (lane 1), since a *Hin*dIII site was not regenerated at the end of the *URA3* gene in the *gal11* locus. Instead, an E fragment larger than the D fragment was obtained by cleavage at the GATC sites, one in *URA3* and the other in the *gal11* locus (*Dpn*I and *Sau*3AI panels, lanes 1). The signals detected between fragments A and C in *Dpn*I-cleaved samples and between fragments C and D in *Mbo*I-cleaved samples may be partial digests of fragment A. In the *Dpn*I panel, a *gal11*Δ mutation caused an increase in fragment

TABLE 4. Effects of a *gal11*Δ mutation on the 5-FOA resistance of strains having *URA3* at telomeric and nontelomeric loci

Strain	Location of *URA3*[a]	*GAL11* locus	5-FOA resistance[b] (range)
YS92	Normal locus (V)	*GAL11*$^+$	$<1 \times 10^{-6}$
YS99	Normal locus (V)	*gal11*Δ	5.3×10^{-6}
YS94	Telomere (VII-L)	*GAL11*$^+$	0.46 (0.37–0.56)
YS95	Telomere (VII-L)	*gal11*Δ	7.5×10^{-4} (4×10^{-4}–9×10^{-4})

[a] The roman numerals designate which chromosome carries the experimental *URA3* locus, and L indicates that *URA3* is on the left arm of the chromosome.
[b] Determined from at least four independent experiments as described in Materials and Methods and expressed as the average ratio of colonies formed on synthetic complete medium containing 5-FOA to those formed on synthetic complete medium.

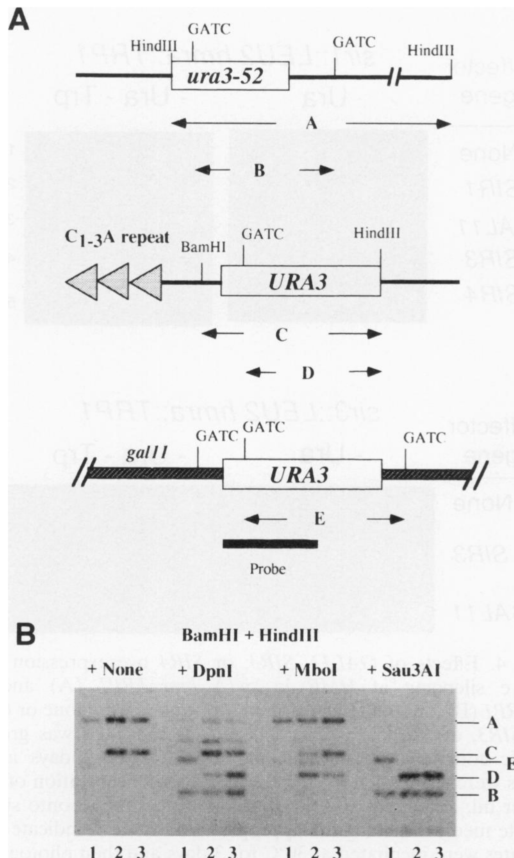

FIG. 2. Effect of a *gal11*Δ mutation on in vivo methylation of the TEL-*URA3*. (A) Partial restriction map of the *ura3-52*, TEL-*URA3*, and *gal11*Δ::*URA3* loci. Note that the map is not drawn to a scale. GATC stands for the GATC site susceptible to *dam* methylation. A black bar indicates a *Sma*I-*Eco*RV fragment used as a probe. Fragments to be generated by restriction enzyme cleavage (A to E) are indicated. (B) Southern analysis of DNA from YS93 (*gal11*Δ::*URA3*; lane 1), YS94 (*GAL11*$^+$ TEL-*URA3*; lane 2), and YS95 (*gal11*Δ::*LEU2* TEL-*URA3*; lane 3) strains expressing *E. coli dam* methylase from a YCp plasmid. DNA was first cleaved with *Bam*HI and *Hin*dIII and then digested with *Dpn*I, *Mbo*I, or *Sau3*AI or not subsequently digested as indicated. The fragments depicted in panel A are indicated on the right. The band between fragments A and C in the *Dpn*I panel and that between fragments C and D in the *Mbo*I panel (lane 2) may correspond to the partial digests of fragment A; the former was cut at the GATC site in the *ura3-52* locus, and the latter was cut at the site to the right of the *ura3-52* locus.

B and a decrease in the fragment between fragments A and C (lanes 2 and 3), suggesting that the accessibility to the methylase of the GATC site at the right-hand side of the *ura3-52* locus was also affected by the mutation. Note that this site is not in the *URA3* gene but is in the Ty sequence whose insertion generated the *ura3-52* allele.

Interaction of *GAL11* with *SIR3* at telomeres. The fact that *GAL11* is involved both in the maintenance of the normal length of the $C_{1-3}A$ repeat and in the telomere position effect prompted us to test the interaction of *GAL11* with *SIR3* and *SIR4*, which are known to affect the telomere position effect (1). We examined whether overexpression of *GAL11* in a *sir3*Δ or *sir4*Δ strain restores transcriptional repression at telomeres; the converse experiment was also done. The *sir1*Δ mutation was not tested, since *SIR1* is not involved in the telomere position effect (1). We assayed the telomere position effect by plating serial 10-fold dilutions of the respective transformants. When this method was used with strains having *URA3* at telomeric versus nontelomeric loci, the results shown in Fig. 3A coincided well with those in Table 4. Overproduction of SIR3 in *gal11*Δ cells could completely suppress the defect in repression of *URA3* at telomeres (Fig. 3B, row 3). Introduction of *SIR3* in a YCp plasmid, however, failed to restore repression of TEL-*URA3* (data not shown). Overproduction of GAL11 could not suppress expression of TEL-*URA3* in *sir3*Δ or *sir4*Δ cells (Fig. 3B, rows 5 and 8), while it could restore repression in *gal11*Δ cells (row 2). Hyperproduction of SIR3 also failed to restore repression in *sir4*Δ cells (row 9). These results indicate that *GAL11*, *SIR3*, and *SIR4* are all involved, though to different extents, in the telomere position effect and in chromatin structure near telomeres. The differences in the efficiency of derepression of TEL-*URA3* in different mutants and in multicopy suppression imply that *SIR3* is more directly involved in the telomere position effect than *GAL11* is.

Since *SIR3* is a limiting factor of the telomere position effect (37), the apparent *GAL11* effect on the structure and function of telomeres may be exerted through the dosage effect by *SIR3*; that is, a *gal11*Δ mutation causes a reduction in *SIR3* expres-

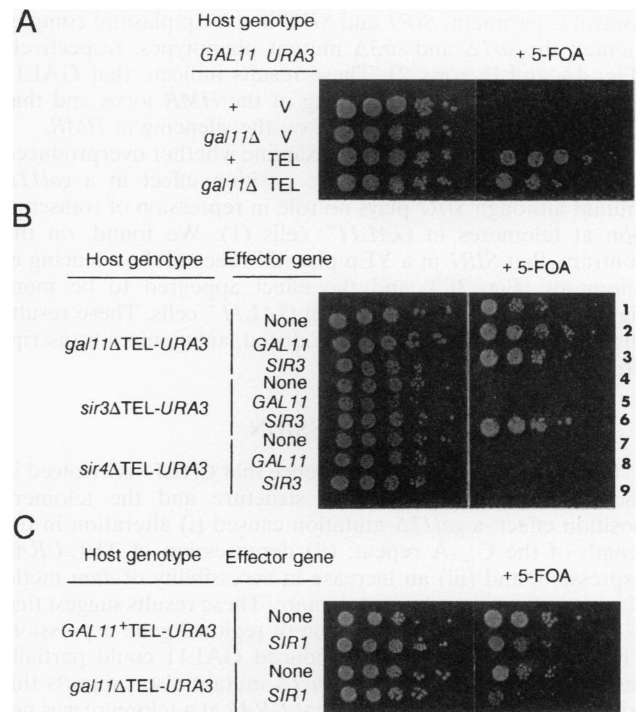

FIG. 3. Effect of a *gal11*Δ mutation (A) and effects of overexpression of *GAL11* or *SIR3* (B) and of *SIR1* (C) on *URA3* expression of either telomeric or nontelomeric location. (A) Strains from the top are YS92 (*GAL11 URA3*), YS99 (*gal11*Δ *URA3*), YS94 (*GAL11* TEL-*URA3*), and YS95 (*gal11*Δ TEL-*URA3*). Strains YS95 (*gal11*Δ), YS97 (*sir3*Δ), and YS98 (*sir4*Δ) (B) and strains YS94 and YS95 (C) were tested for TEL-*URA3* expression when they carry a YEp plasmid as indicated. Colonies of each strain grown on rich medium (A) or synthetic complete medium minus Trp (B and C) for 3 days at 30°C were suspended in sterile water at a concentration of ca. 10^8 cells per ml, and serial 10-fold dilutions were plated onto synthetic complete medium with or without 5-FOA (A) or synthetic complete medium minus Trp with or without 5-FOA (B and C) as described in Materials and Methods. After 3 days at 30°C, plates were photographed.

sion. We tested this possibility by measuring the amount of *SIR3* message by Northern (RNA) analysis and found that there was no significant difference in the level of *SIR3* expression between *GAL11*$^+$ and *gal11*Δ cells (data not shown).

GAL11* and the silencing of the *HMR*a locus.** Since *SIR3* and *SIR4* were originally identified as genes required for maintenance of transcriptional repression of the *HMR* and *HML* loci (38), we tested whether *GAL11* is also involved in silencing at the *HM* loci. Our previous report demonstrated that *HMR* information is not expressed in *MAT*α *gal11*Δ cells (35), which makes it unlikely that a *gal11*Δ mutation derepresses *HMRa**. However, it is possible that *GAL11* plays a minor role in the silencing at *HMR* such that *HMR***a** was derepressed to a level undetected by our Northern analysis. Alternatively, the silencing mechanism may be redundant, including a *GAL11*-independent mechanism. Therefore, we constructed a Δ*hmr*::*TRP1* disruption (48) in *sir1*Δ and *sir3*Δ strains and tested whether multiple copies of *GAL11* in these strains can restore repression of *TRP1* expression. Overexpression of *GAL11* partially restored silencing of the *HMR* locus in the *sir1*Δ mutant (Fig. 4A, row 3) but failed to do so in the *sir3*Δ mutant (Fig. 4B, row 3). Hyperproduced GAL11 also failed to suppress expression of *TRP1* in the *HMR* locus in *sir4*Δ cells (data not shown). Overexpression of *SIR3* could restore the silencing in *sir1*Δ cells more efficiently than *GAL11* could, whereas *SIR4* in a YEp plasmid failed to do so (Fig. 4A, rows 4 and 5). In a control experiment, *SIR1* and *SIR3* in a YEp plasmid complemented the *sir1*Δ and *sir3*Δ mutant phenotypes, respectively (Fig. 4A and B, rows 2). These results indicate that GAL11 may be involved in the silencing of the *HMR* locus and that *SIR3* also exerts a dosage effect on the silencing at *HMR*.

This finding prompted us to examine whether overproduced SIR1 can restore the telomere position effect in a *gal11*Δ mutant although *SIR1* plays no role in repression of transcription at telomeres in *GAL11*$^+$ cells (1). We found, on the contrary, that *SIR1* in a YEp plasmid relieved the silencing at telomeres (Fig. 3C), and the effect appeared to be more significant in *gal11*Δ cells than in *GAL11*$^+$ cells. These results suggest that SIR1, when overproduced, antagonizes transcriptional repression at telomeres.

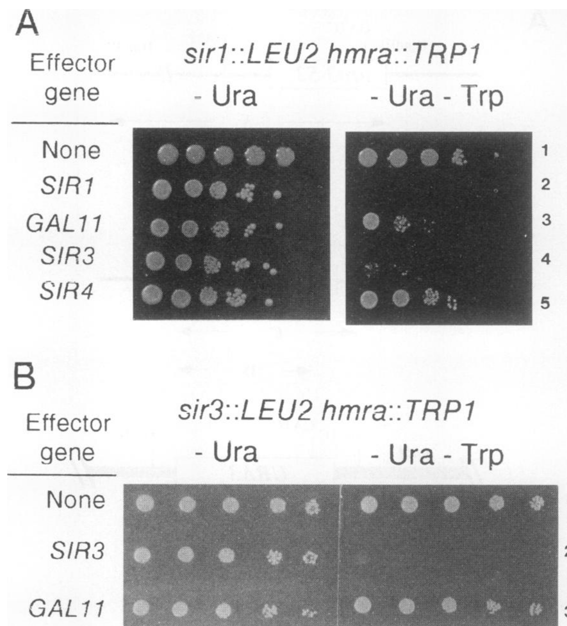

FIG. 4. Effects of *GAL11*, *SIR3*, or *SIR4* overexpression on the defective silencing at *HMR* in *sir1*Δ *hmr*::*TRP1* (A) and *sir3*Δ *hmr*::*TRP1* (B) mutants. Each strain harboring vector alone or *GAL11*, *SIR1*, *SIR3*, or *SIR4* in a YEp plasmid as indicated was grown on synthetic complete medium lacking uracil. After 2 days at 30°C, colonies were suspended in sterile water at a concentration of ca. 10^8 cells per ml, and serial 10-fold dilutions were spotted onto synthetic complete medium lacking the appropriate nutrient as indicated at the top. Plates were incubated at 30°C for 3 days and then photographed.

DISCUSSION

In this report, we present evidence that *GAL11* is involved in the maintenance of telomere structure and the telomere position effect: a *gal11*Δ mutation caused (i) alteration in the length of the $C_{1-3}A$ repeat, (ii) derepression of TEL-*URA3* expression, and (iii) an increase in accessibility of *dam* methylase to a gene placed near telomere. These results suggest that *GAL11* plays a role in regulation of regional gene expression. The observation that hyperproduced GAL11 could partially restore silencing at *HMR* in a *sir1*Δ mutant also supports this conclusion. However, the fact that *URA3* at a telomere was not fully derepressed by a *gal11*Δ mutation implies that GAL11 is an auxiliary factor for the position effect and that an additional factor(s) has to be removed for full derepression. Similarly, a *gal11*Δ mutation alone was not sufficient to render the GATC site near a telomere fully susceptible to *dam* methylation (Fig. 2). Alternatively, *GAL11* is required for TEL-*URA3* expression as well as repression, since GAL11 is required for expression of *URA3* at its native locus (16), and Renauld et al. showed that the strength of a gene's promoter is a major determinant for transcriptional repression at telomeres (37). Nevertheless, the fact that *URA3* at a telomere was expressed to a significant level in a *gal11*Δ mutant indicates that *GAL11* does not play a major role in transcriptional activation of TEL-*URA3*. In other words, when closed chromatin structure at a telomere is relieved, TEL-*URA3* transcription can be activated in the absence of *GAL11* (discussed below).

Although the different effects of a *gal11*Δ mutation on *SUC2* and *SUC7* expression led us to initiate this work that has revealed the involvement of GAL11 in regulation of regional gene expression, the effect of a *gal11*Δ mutation on *SUC7* expression may not be explained solely by the telomere position effect. The *SUC2* and *SUC7* promoters may respond differently to *GAL11*: under derepressing conditions, the two promoters in a plasmid showed distinct sensitivity to loss of GAL11 (Table 3). In spite of the conservation of their upstream sequences (up to −500 for *SUC2*), including the upstream regulatory region for *SUC2* expression, the two sequences become diverged beyond −500 (41). In this region, *SUC2* has a binding site for SKO1, one of the factors necessary for glucose repression of *SUC2*, at −627, whereas *SUC7* does not (32, 41). It is possible that *GAL11* negatively regulates *SUC7* at its normal locus by a mechanism yet to be identified. It also should be noted that *SUC7* is located at least ca. 11 kbp from the terminus of a chromosome (4) and that *URA3* at a distance of 10 kbp from the telomere of the right arm of chromosome V was not repressed (37). Although the strength of repression varies with different chromosomal environments (*SUC7* is embedded in the telomere-associated X and Y′ sequences, whereas the right arm of chromosome V lacks the telomere-associated sequence X [4, 14, 37]), more experiments are needed to determine whether *SUC7* is under the telomere position effect.

A number of proteins, including RAP1, SIR2, SIR3, SIR4, histone H4, NAT1, and ARD1, affect the structure of and the

position effect at telomeres (1, 24, 28, 36, 48). RAP1 binds to the telomere $C_{1-3}A$ repeat (3, 8, 45), and mutations in *RAP1* cause an alteration, either a lengthening or diminution, in the length of the $C_{1-3}A$ repeat (8, 28, 48). Recently, Kyrion et al. reported that the C-terminal portion of RAP1 is responsible for regulation of the structure and the position effect of telomeres (24, 25). These functional similarities of GAL11, RAP1, and SIR proteins raise the possibility that they act cooperatively in the maintenance of the structure and function of telomeres. However, their contributions appear to be different: the *rap1-17* allele exhibits a drastic alteration in the length of the $C_{1-3}A$ repeat (700 to 2,000 bp [25]), whereas a *gal11*Δ mutation caused a modest alteration (120 bp; Fig. 1). Both *rap1-17* and *sir3*Δ mutations relieve the silencing at telomeres fully and alter chromatin structure near telomeres drastically (25), while a *gal11*Δ mutation did so partially (Table 4, Fig. 2, and Fig. 3). And overproduction of SIR3 could suppress expression of TEL-*URA3* in a *gal11*Δ mutant (Fig. 3). These results suggest that RAP1 and the SIR proteins function as a core complex in repression and that GAL11 is an auxiliary factor of the complex. SIR3 and SIR4 have been shown genetically to interact with each other and exert a dosage effect in silencing (26, 29, 49; this work). GAL11 may function by helping assembly of the complex. This GAL11 function can be explained by either protein-protein interaction or alteration in chromatin structure. Although there is no direct evidence for physical interaction between GAL11 and factors involved in the position effect, genetic evidence suggests their interaction (35; this work). Alternatively, GAL11, when combined with RAP1 and SIR proteins, may alter chromatin structure to aid the assembly of the repression complex and/or to establish closed chromatin at telomeres.

Transcriptional repression mechanisms at telomeres and at silencers are similar, involving common factors (1) and chromatin structure (13, 22, 31, 46). In our previous report, however, *GAL11* did not appear to be involved in silencing at the *HM* loci; no derepression of *HMR***a** information was observed in *MAT*α *gal11*Δ cells, and a *MAT***a** *gal11*Δ strain mates normally (35). Thus, *GAL11* and *SIR1* appear to function reciprocally; *SIR1* is involved in the *HM* silencing but not in the telomere position effect (1, 38). Nevertheless, *GAL11* and *SIR1* exhibit similarities in their functional roles. For example, *gal11*Δ mutation does not lead to full derepression of TEL-*URA3*, just as a *sir1*Δ mutation does not fully relieve silencing of the *HM* loci (reference 38 and this work). Overproduced GAL11 and SIR1 failed to restore the position effect at telomeres (GAL11) and silencing at the *HM* loci (SIR1) in a *sir3*Δ mutant (47; this work). We imagine that at telomeres, GAL11 fulfills a role like the role that SIR1 plays in transcriptional repression at the silencers of the *HM* loci. In this process, SIR1 is suggested to act as "glue" (47) which can be replaced in part by hyperproduced GAL11. The observation that hyperproduced SIR3 could suppress defects in silencing both at a telomere by Δ*gal11* and at *HMR* by Δ*sir1* supports this hypothesis. However, the effects of overexpressed GAL11 and SIR1 were distinct; the former could partially compensate for the loss of SIR1 in the *HMR* silencing, whereas the latter relieved the position effect at telomeres. This finding suggests an alternative model in which two mechanisms, *SIR1* dependent and independent, are involved in silencing at *HMR*. The results from genetic studies described above are also consistent with this model. Both mechanisms require the other SIR proteins, and the *SIR1*-independent pathway may be shared with the silencing at telomeres, using GAL11 as an auxiliary factor. According to this model, a *gal11*Δ mutation does not affect the *SIR1*-dependent pathway and therefore does not appear to alter the silencing state at the *HM* loci. Overexpression of *GAL11* would stimulate the *SIR1*-independent pathway, resulting in partial restoration of silencing at *HMR* in the absence of *SIR1*, whereas excess SIR1 protein might perturb the assembly of the repression complex at telomeres (by titrating out its components?), especially in the absence of GAL11. Recently, Chien et al. (7) showed that the telomere position effect can be enhanced by tethering a GAL4 DNA-binding domain–SIR1 chimeric protein to the telomere and concluded that the unstability of the position effect at telomeres is caused by the inability of telomeres to recruit the SIR1 protein. According to their model, SIR1 plays a central role in establishing the transcriptionally silent state by recruiting other SIR proteins or by modifying the function of SIR proteins. We assume that GAL11 also can help the assembly of the repression complex at telomeres, though less efficiently than the DNA-bound SIR1 can.

GAL11 is required for accurate regulation of transcription of a diverse set of genes (10, 16, 35, 50). At promoters where GAL11 functions positively, GAL11 may help DNA-bound activators to assemble and/or stabilize the initiation complex (16, 35). It is possible that GAL11 itself can interact with the basal factors, since it can activate transcription when tethered to DNA (16, 35a). These functions, however, can be carried out through alteration in chromatin structure at promoters, since nucleosomes are inhibitory to the assembly of the initiation complex. Therefore, we propose that GAL11, like the SNF2-SNF5-SNF6 complex (27), plays roles both in alteration of chromatin structure and in stimulation of the transcription machinery and that GAL11 function, either positive or negative, is determined by a combination of DNA-bound factors and the structures of the target promoters (35a). When GAL11 is combined with RAP1 (and other factors), it functions as a negative regulator at telomeres and as a positive factor at the *PYK1* promoter (35). For TEL-*URA3* expression, GAL11 may act both positively in conjunction with PPR1 (16) and negatively by maintaining the closed chromatin structure with RAP1 and SIR proteins.

One may argue, however, that GAL11's effect is indirect: the alteration in chromatin structure at TEL-*URA3* in a *gal11*Δ mutant may be caused by transcription of *URA3*, since transcriptional activation is accompanied with displacement of nucleosome, or *GAL11* affects expression of genes encoding factors involved in the position effect at telomeres. It is difficult to think of a mechanism by which loss of GAL11 directly activates the *URA3* promoter, since *GAL11* is required for full transcriptional activation of *URA3* by PPR1 (16). We cannot, however, rule out the possibility that transcription of an unknown gene adjacent to TEL-*URA3* is activated by a *gal11*Δ mutation, resulting in an alteration in the chromatin structure and a consequent expression of TEL-*URA3*. Also, it is still possible that *GAL11* affects expression of a gene encoding a limiting factor other than SIR3, although *SIR3* expression is not regulated by GAL11.

GAL11 plays a role in transcriptional regulation as an auxiliary factor at both promoters and telomeres and is likely to function in conjunction with DNA-bound factors. Although the presence of such a factor may add more complexity to the regulation mechanism of transcription, its identification forms another connection between structure and biological functions of DNA, including transcription, replication, and recombination.

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