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# A class of single-stranded telomeric DNA-binding proteins required for Rap1p localization in yeast nuclei

**(telomeres/nuclear organization/heteronuclear ribonucleoprotein)**

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**ABSTRACT** **We have identified a class of proteins that bind single-stranded telomeric DNA and are required for the nuclear organization of telomeres and/or telomere-associated proteins. Rlf6p was identified by its sequence similarity to Gbp1p, a single-stranded telomeric DNA-binding protein from *Chlamydomonas reinhardtii*. Rlf6p and Gbp1p bind yeast single-stranded G-strand telomeric DNA. Both proteins include at least two RNA recognition motifs, which are found in many proteins that interact with single-stranded nucleic acids. Disruption of *RLF6* alters the distribution of repressor/activator protein 1 (Rap1p), a telomere-associated protein. In wild-type yeast cells, Rap1p localizes to a small number of perinuclear spots, while in *rlf6* cells Rap1p appears diffuse and nuclear. Interestingly, telomere position effect and telomere length control, which require *RAP1*, are unaffected by *rlf6* mutations, demonstrating that Rap1p localization can be uncoupled from other Rap1p-dependent telomere functions. In addition, expression of *Chlamydomonas GBP1* restores perinuclear, punctate Rap1p localization in *rlf6* mutant cells. The functional complementation of a fungal gene by an algal gene suggests that Rlf6p and Gbp1p are members of a conserved class of single-stranded telomeric DNA-binding proteins that influence nuclear organization. Furthermore, it demonstrates that, despite their unusual codon bias, *C. reinhardtii* genes can be efficiently translated in *Saccharomyces cerevisiae* cells.**

Telomeres, the DNA–protein complexes at the ends of linear chromosomes, stabilize and protect the chromosomal termini (reviewed in refs. 1 and 2). Chromosomes often appear highly organized within the nucleus, with the telomeres clustered at the nuclear periphery (3). Telomere-associated proteins are thought to participate in telomere replication, the protection of telomeric DNA from degradation, and the associations between telomeres and other nuclear structures. Telomeric DNA from many organisms is composed of short repeat sequences that include multiple guanines and few, if any, cytosines on the strand that forms the 3′ end of the chromosomal DNA (G-strand) (reviewed in ref. 4).

In *Saccharomyces cerevisiae*, Rap1p binds double-stranded (ds) telomeric DNA *in vitro* (5–7) and *in vivo* (8–10) and is required for telomere length control (11) and telomere position effect (TPE) (12), the transcriptional silencing of genes placed close to the chromosome ends (13). Rap1p also binds to single-stranded (ss) yeast telomeric G-strand DNA, albeit with a much lower affinity than it binds to ds telomeric DNA (14).

In pachytene spreads, Rap1p localizes primarily to telomeres (15), and, in interphase, Rap1p localizes to a small number of perinuclear spots (15) that colocalize with telomeric DNA (H. Scherthan, T. Laroche, and S. Gasser, personal communication). Mutations in genes that alter telomere length (11) and TPE (12) also change the localization of Rap1p from punctate and perinuclear to diffuse and nuclear (11). Taken together, these results suggest that wild-type, interphase telomeres associate with Rap1p and with one another primarily at the nuclear periphery (3, 11, 15). While Rap1p binds ds telomeric DNA, chromosomal termini are characterized by short ssDNA overhangs (16, 17).

A number of proteins that bind ss G-strand telomeric DNA have been characterized. In hypotrichous ciliates, telomeric protein–DNA complexes are resistant to high salt and DNA in the complexes is protected from exonucleolytic degradation (18, 19). The ciliate telomere-binding proteins bind 3′ overhang sequences and share a high degree of similarity (20–23). Like the ciliate telomere-binding proteins, a *Xenopus* egg protein, X-TEF, binds specifically to vertebrate 3′ overhang telomere sequences *in vitro* (24). Several vertebrate proteins that bind *in vitro* to ss G-strand telomeric DNA have also been identified. These include heteronuclear ribonucleoproteins (hnRNPs) A2/B1, A1, D, and E (25–27) as well as lamins and vimentin (28). Because the hnRNPs appear to have a much higher affinity for r(UUAGGG)$_n$ RNA substrates than for the cognate telomeric DNA sequence d(TTAGGG)$_n$, the role, if any, of hnRNPs at telomeres is not clear (25, 27).

Gbp1p is a *Chlamydomonas reinhardtii* protein that binds the ss G-strand *Chlamydomonas* telomeric DNA sequence, (TTTTAGGG)$_n$, as ss DNA and as a 3′ overhang structure (29, 30). Like many hnRNPs, Gbp1p includes two RNA recognition motifs (RRMs) (29), domains characterized by an ≈80-amino acid region containing a highly conserved RNP consensus octamer (31, 32) or RNP-1 (33). The RNP-1 consensus is found in many proteins (including hnRNPs) that bind RNA and ss DNA (26, 27, 33–36). Unlike the vertebrate hnRNPs, Gbp1p does not bind cognate telomeric RNA specifically (29). In this paper we demonstrate that *RLF6*, a yeast gene identified by its sequence similarity to *GBP1*, is required for the appropriate nuclear localization of Rap1p. Furthermore, *GBP1* can be expressed in yeast cells where it functionally complements the Rap1p localization defect of *rlf6* mutants.

## METHODS

**Sequence Analysis.** Predicted open reading frames Gbp1p (GenBank accession no. U10442) and Rlf6p/YCL11c (SwissProt accession no. P25555) were compared using FASTA and the two proteins were aligned using COMPARE and DOTPLOT in the Genetics Computer Group suite of programs (37).

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Abbreviations: ss, single-stranded; ds, double-stranded; hnRNP, heteronuclear ribonucleoprotein; RRM, RNA recognition motif; TPE, telomere position effect; DAPI, 4′,6-diamidino-2-phenylindole.

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For Gbp1p and Rlf6p, the FASTA optimized similarity score is 246. The next highest scores were 219 and 192 for a maize gene induced by abcisic acid and a *Drosophila* poly(A)-binding protein, respectively. Sequence comparisons were done with a window of 30 and a stringency value of 15.

**Strains, Plasmids, and Oligonucleotides.** The *Escherichia coli* strains DH5-α (*endA1 hsdR17* ($r_K-m_{K+}$) *supE44 thi-1 recA1 gyrA relA1* Δ(*lacZYA-argF*) *U169 deoR* [ϕ80*dlac*Δ(*lacZ*)*M15*] and XL1-blue (Stratagene) were used as the host strains for plasmid propagation. *E. coli* RR1 (38) was used for expression of the TrpE-Gbp1p fusion protein. Yeast strains are listed in Table 1. Oligonucleotides YG3 (5′-TGTGTGGGTGTGTGGGTGTGT-GGG) and CG3 (5′-TTTTAGGGTTTTAGGGTTTTAGGG) were synthesized on a Pharmacia Geneassembler, deprotected, and end-labeled with [γ-$^{32}$P]ATP and T4 polynucleotide kinase (Promega) according to manufacturer's instructions. Oligonucleotides were purified on a 20% polyacrylamide/7 M urea gel (40) prior to use in gel retardation or Southwestern binding assays. All ss oligonucleotides were denatured by boiling for 5 min followed by incubation on ice. Yeast strains were grown in SD medium (41) containing either 2% glucose or 2% galactose, as indicated, and all amino acids and nucleotides other than leucine and/or uracil as appropriate for plasmid selections. Deletion disruption alleles of the 2.9-kb *Eco*RI–*Hin*dIII fragment of *RLF6* were generated using Mini-Tn*3* transposons m-Tn*3* (*LEU*) or m-Tn*3* (*URA*) in shuttle mutagenesis (42). Plasmids carrying the disruption alleles were used to replace a wild-type copy of *RLF6* in a YJB252 × YJB203 diploid by one-step gene replacement (43). Diploid transformants were sporulated and haploid strains carrying appropriate markers and *rlf6* disruption alleles (YJB773, YJB776, YJB781, and YJB783, Table 1) were isolated and the positions of the inserted markers were determined by restriction mapping. Isogenic strains carrying *URA3* on chromosome VIIL were constructed by transformation with plasmid VIIL-*URA3*-TEL, which inserts within the *ADH4* locus and replaces sequences distal to *ADH4* with *URA3* and a telomere fragment (13). TPE assays were performed on five independent transformants per strain (13).

**Southern Blots, Protein Extracts, and Binding Assays.** Cells were grown in complete medium for at least 25 generations and genomic DNA was digested with *Xho* I, which cleaves 870 bp from the junction between the Y′ repeat and the terminal $TG_{1-3}$ tract. The $TG_{1-3}/C_{1-3}A$ probe was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) incorporated using the PCR and universal primers (44) to amplify the 72-bp $TG_{1-3}/C_{1-3}A$ from plasmid pCA75 (45).

*Chlamydomonas* cells were harvested and extracts were prepared (29). Yeast cells were harvested and disrupted following essentially the same protocol except that cells were spun in a Vortex with glass beads for 3 min per cycle with incubation on ice for 4 min between cycles for three or four cycles, until >70% of cells were disrupted. *E. coli* cells were transformed with pTL5 (29), which encodes a TrpE-Gbp1p fusion protein that includes the entire *GBP1* cDNA, and extracts were prepared as described. Southwestern blot analysis and gel retardation assays were performed as described using $^{32}$P-end-labeled oligonucleotides (29). Immunoblots were performed as described using affinity-purified anti-PEPG1 antibody raised against a Gbp1p peptide (29).

Table 1. Yeast strains used

| Strain | Genotype | Source or ref. |
|---|---|---|
| YJB203 | *Mat***a** *leu2 ura3 his3 ade2 CYH$^s$* [*cir0*] | 39 |
| YJB252 | *Matα leu2 ura3 his3 ade2 cyh2$^r$* [*cir0*] | This study |
| YJB773 | YJB252 *cyh2$^r$ rlf6::URA3-A3* | This study |
| YJB776 | YJB252 *cyh2$^r$ rlf6::URA3-A10* | This study |
| YJB781 | YJB203 *cyh2$^r$ rlf6::LEU2-B4* | This study |
| YJB783 | YJB203 *cyh2$^r$ rlf6::LEU2-B7* | This study |
| YJB917 | YJB203 *adh4::URA3-tel* | This study |
| YJB918 | YJB783 *adh4::URA3-tel* | This study |
| YJB860 | YJB781 [pGAL10-GBP1] | This study |
| YJB862 | YJB783 [pGAL10-GBP1] | This study |
| YJB868 | YJB781 [pBM272] | This study |
| YJB894 | YJB203 [pBM272] | This study |
| YJB895 | YJB203 [pGAL10-GBP1] | This study |

**Indirect Immunofluorescence Microscopy.** Immunofluorescence methods were performed using standard protocols (46). Yeast cells were grown in the medium indicated in the figure legend and fixed in 5% formaldehyde for 90 min, and cell walls were digested with 50 μg of Zymolyase per ml (ICN) plus 50 μl of Glusulase per ml (DuPont). Cells were washed three times in TBS (5 mM Tris·HCl, pH 8.0/75 mM NaCl); incubated with blocking solution [10 mg of bovine serum albumin per ml (Sigma), 0.5% Nonidet P-40 (Sigma), in TBS] for 10 min; incubated with affinity-purified anti-PEP1 (1:20), a rabbit polyclonal antibody raised against a Rap1p peptide (7); washed three times with TBST (TBS/0.5% Tween 20/0.1% $NaN_3$); and incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch). DNA was counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and mounted in DABCO anti-fade solution (24.5 mg of diazabicyclo-2′,2′,2′-octane in 75% glycerol). Cells were observed and photographed on a Zeiss Photomicroscope III using fluorescein and DAPI filter sets.

## RESULTS

**Gbp1p and Rlf6p Have Similar Domain Structures.** Sequence comparison of Gbp1p to predicted proteins in the public databases revealed that YCL11c, an open reading frame of unknown function on chromosome III of *S. cerevisiae* (47), predicts a protein with the highest sequence similarity to Gbp1p. We have named YCL11c *RLF6* (Rap1p localization factor 6) for reasons described below. Rlf6p includes three regions similar to the Gbp1p RRMs and two regions similar to the Gbp1p (Arg + Gly)-rich region (Fig. 1*A*).

RRMs include a highly conserved octad sequence termed the RNP-1 region (31). Interestingly, in all five of the predicted RRMs in Gbp1p and Rlf6p, the amino acid at the fifth position of this octad does not conform to the conserved sequence, which is either a phenylalanine or a tyrosine in almost all other RRM proteins studied (31) (Fig. 1 *C*). In addition, the RRMs in Tom34p, a predicted protein that contains RRMs with a high degree of similarity to Rlf6p (48), and in Nsr1p, a protein that binds G-strand DNA (51), also do not conform to the RRM consensus sequence in the fifth position of RNP-1 (Fig. 1*C*).

**Phenotypes of *rlf6* Mutants.** To study Rlf6p function, we generated yeast strains carrying *rlf6* disruption alleles (Table 1, Fig. 1*B*). Haploid strains carrying *rlf6* disruption alleles exhibited no obvious growth defects at 12–37°C on a variety of carbon sources (P.M.-Z., L.M.C.K., and M. Epshteyn, unpublished data). We also analyzed the properties of telomeres in *rlf6* mutants. There was no significant difference in the average length of terminal telomere repeat tracts from wild-type and isogenic *rlf6* strains (Fig. 2*A*). TPE was assayed in YJB918, a strain carrying the *rlf6::LEU2-B7* disruption allele (Fig. 1*B*). YJB917 (wild-type) and YJB918 (*rlf6::LEU2-B7*) grew in a similar manner with close to 100% of the cells able to grow in the absence of uracil and ≈5% of the cells able to grow on 5-fluoroorotic acid (Table 2). Thus, *RLF6* is not required for yeast cell growth, telomere tract length control, or TPE, the maintenance of transcriptional repression at telomeres.

**G-Strand Binding Activities of Rlf6p and Gbp1p.** We asked whether Rlf6p, like Gbp1p, binds ss telomeric DNA using a yeast G-strand oligonucleotide (YG3). In wild-type yeast cells, YG3-binding activities are observed in Southwestern blots

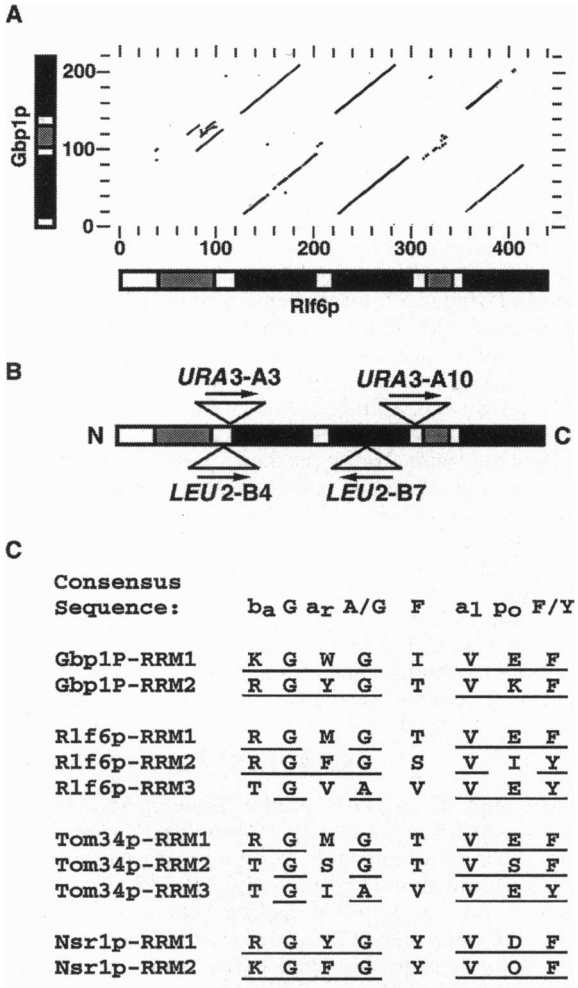


FIG. 1. Structure of Gbp1p, Rlf6p, and *rlf6* disruption alleles. (*A*) Sequence similarities between the large open reading frames in *GBP1* (left) and in *RLF6* (bottom). Black boxes, predicted RRMs (31); gray boxes, regions rich in Arg and Gly. (*B*) Structure of disruption alleles of *RLF6*. RRMs and (Arg+Gly)-rich sequences are illustrated as in *A*. Arrows indicate the direction of transcription of inserted genes. (*C*) Regions of Gbp1p and Rlf6p that line up with the RNP-1 consensus pattern as described (31). The RNP-1 regions of RRMs in Gbp1p, Rlf6p, Tom34p (48), and Nsrlp (49) are shown. Positions highly conserved in other RRMs are underlined. Amino acid groups (50) are designated in lowercase letters: ba, basic; ar, aromatic; al, aliphatic; po, polar. Uppercase letters, standard amino acid abbreviations.

(Fig. 2*B*, lane 1). An ≈68-kDa YG3-binding activity present in wild-type cells is missing in cells carrying disrupted *rlf6* alleles (Fig. 2*B*, lane 2). Thus, disruption of *RLF6* correlates with a loss of G-strand binding activity of appropriate size. Similar results were observed with all four *rlf6* alleles (Fig. 1*B* and data not shown).

**Immunolocalization of Rap1p in *rlf6* Mutant Strains.** In wild-type interphase cells, Rap1p appears as a small number of brightly staining spots (Fig. 3, top panels), when localized by indirect immunofluorescent microscopy. This is consistent with the punctate, perinuclear Rap1p pattern seen by others (3, 11, 15) using similar methods with different anti-Rap1p antibodies. In contrast, in strains carrying *rlf6* disruption alleles (Fig. 1*B*), Rap1p appears diffuse and nuclear (Fig. 3, second panels), indicating that Rlf6p is required for appropriate Rap1p localization. For this reason, we have named YCL11c Rap1 localization factor 6. Five other yeast *RLF* genes, *RLF1–RLF5*, have been identified genetically (S.E. and J.B., unpublished data).

**Expression of Gbp1p in *rlf6* Mutant Strains.** *Chlamydomonas* Gbp1p, in *C. reinhardtii* extracts or expressed in *E. coli* as a TrpE-fusion protein, binds yeast telomeric DNA, albeit with a lower affinity than it binds *Chlamydomonas* telomeric DNA (Fig. 2*B*, lanes 3–6; L.M.C.K., unpublished data). To ask whether Gbp1p and Rlf6p have similar functions, we expressed *GBP1* under the control of the yeast *GAL10* promoter (YJB860 and YJB862, Table 1) in *rlf6::LEU2* disruption strains. Gbp1p was expressed from pGAL10-GBP1 in yeast cells grown on galactose (Fig. 2*B*, lane 9), but not on glucose (Fig. 2*B*, lane 8), and it had the same apparent molecular weights on SDS/polyacrylamide gels when expressed in *Chlamydomonas* extracts or in yeast cells. Furthermore, Gbp1p expressed in yeast bound YG3 in Southwestern blot assays (Fig. 2*B*, lane 7) and in gel mobility shift assays (Fig. 2*C*).

Since Gbp1p expressed in yeast is able to bind yeast telomeric DNA, we asked whether *GBP1* expression can restore Rap1p localization in *rlf6* mutants. Rap1p remained nuclear and diffuse (Fig. 3, third panels) in YJB860 and YJB862 grown on glucose (repressing conditions). Interestingly, when these strains were grown on galactose (inducing conditions), Rap1p localized in a punctate pattern similar to Rap1p in wild-type cells (Fig. 3, bottom panels). Thus, *Chlamydomonas* Gbp1p functionally complements the Rap1p localization defect in yeast cells carrying *rlf6* disruptions.

## DISCUSSION

This study demonstrates that Rlf6p, a protein identified by its similarity to the *Chlamydomonas* ss telomeric DNA-binding protein Gbp1p, is required for the appropriate localization of Rap1p. Since Gbp1p can functionally complement *rlf6* mutations, we propose that Rlf6p and Gbp1p are members of a class of proteins that bind telomeric DNA and are required for the nuclear organization of telomeres and/or telomere-associated proteins. Both proteins bind ss G-strand telomeric DNA sequences and include at least two RRM domains, which are found in many proteins that interact with ss nucleic acids.

Appropriate Rap1p localization requires a number of gene products. *SIR3* and *SIR4*, originally identified as silent mating type information regulator genes (53, 54), are required for punctate, perinuclear Rap1p localization (11). Mutations in *SIR3* or *SIR4* also influence TPE (12), leading to the suggestion that the clustering of Rap1p (and presumably telomeres) is required for the maintenance of a transcriptionally silent chromatin structure at telomeres (3, 11). In contrast, *rlf6* mutations affected Rap1p localization but had no significant effect on TPE, suggesting that the maintenance of TPE is not dependent upon the clustering of Rap1p in the nucleus and demonstrating that these two phenotypes can be uncoupled. Similarly, telomere length control requires functional Rap1p (8, 10, 55), Sir3p, and Sir4p (11). In *rlf6* mutants, telomere tract lengths are not perturbed, implying that telomere length control also is not dependent upon Rap1p clustering and that these two phenotypes can be uncoupled.

*RLF6* is different from other genes that disrupt Rap1p localization in that *rlf6* mutations have no deleterious effect on telomere length control, TPE, or cell growth. One model that may explain this result is based on the assumption that telomeric DNA is associated with a large complex of proteins (*Sir* and *Rlf* proteins) and that this telomeric complex is sensitive to the stoichiometry and/or modification state of these proteins. We envision Rap1p as a major component of the telomeric complex, bound not only to telomeric DNA, but to Sir3p and Sir4p through protein–protein interactions. Interactions between Sir3p, Sir4p, and Rap1p have been demonstrated genetically and biochemically (56–58). In wild-type cells, we assume that many Rap1p epitopes are exposed at each telomeric complex, giving rise to the bright, punctate staining pattern we observe by indirect immunofluorescence micros-

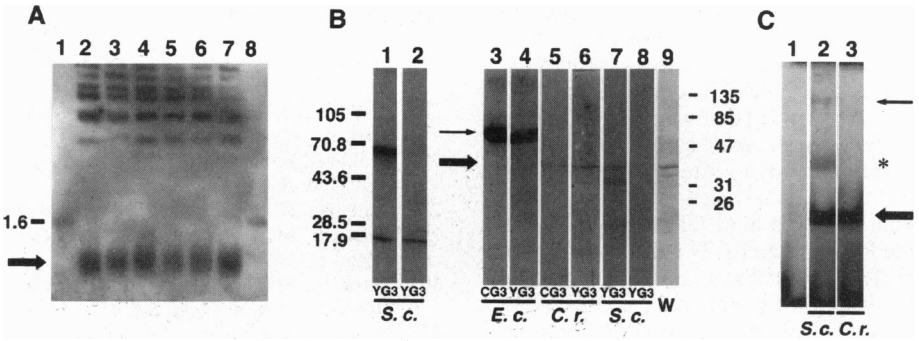


FIG. 2. Telomere length and DNA binding in *rlf6* strains. (*A*) Southern blot of telomere tract length. Lanes 1 and 8, 1.6-kb marker; lanes 2 and 7, YJB203 (wild type); lanes 3–6, *rlf6* disruption strains YJB773, -776, -781, and -783, respectively. Arrow, terminal Y′ fragments. (*B*) Southwestern blot assays (lanes 1–8) using labeled oligonucleotides indicated below the blots and extracts from the following: lane 1, YJB203 (wild type); lane 2, YJB781 (*rlf6::LEU2-B4*); lanes 3 and 4, *TrpE-GBP1* expressed in *E. coli* (29); lanes 5 and 6, *Chlamydomonas* cells; lane 7, YJB860 grown on galactose; lane 8, YJB860 grown on glucose; lane 9, immunoblot (W) of YJB860 grown on galactose (29). Thin arrow, TrpE-Gbp1p (≈65 kDa); wide arrow, intact Gbp1p. (*C*) Gel retardation of Gbp1p. The same 7.5% nondenaturing polyacrylamide gel was loaded with extracts from the following: lane 1, no protein; lane 2, YJB860 grown on galactose; lane 3, *C. reinhardtii* cell extract. Thin arrow, position of a YG3-shifted band that is altered in *rlf6* mutants; asterisk, band with variable intensity in different extracts that does not appear to be related to Rlf6p or to the expression of Gbp1p; wide black arrow, band that appears only in cells expressing *GBP1*. This band is not observed when YJB860 cells are grown on glucose. Unbound $^{32}$P-labeled YG3 appears at the bottom of each lane.

copy. The model proposes that, in *rlf6* strains, the telomeric complex is perturbed such that Rap1p is no longer a prominent epitope for anti-Rap1p antibodies, leading to diffuse staining, yet enough Rap1p remains bound to the ds telomeric DNA to maintain normal telomere length control and TPE levels. Rlf6p could contribute to telomere structure either directly, as a component of the telomeric complex, or indirectly, by modifying the amount or structure of telomeric complex component proteins.

An alternative explanation for the mild effect of *rlf6* mutations on telomere functions may be that additional genes provide functions that are partially or completely redundant with Rlf6p. A candidate for such a gene is *TOM34*, a recently discovered anonymous open reading frame on chromosome XIV that is highly homologous to *RLF6* (47% identical and 87% similar) (48). Interestingly, like the RRMs in Gbp1p and Rlf6p, all three RRMs in *TOM34* diverge from the RNP-1 consensus sequence at position 5 (Fig. 1*C*). Future experiments are necessary to assess the importance of this position in RNP-1 to the binding specificity and Rap1p localization functions of Gbp1p and Rlf6p.

We do not know whether Rlf6p binds RNA or DNA *in vivo* and we do not know whether it colocalizes with telomeres at the nuclear periphery. Since Rlf6p and Rap1p bind telomeric DNA repeats *in vitro*, it is possible that Rlf6p is localized near telomeres *in vivo*. Gbp1p and Rlf6p bind ss telomeric DNA *in vitro* (ref. 29; this work). We also do not know whether Rap1p colocalizes with telomeric DNA in *rlf6* mutant cells. While it is possible that telomeric DNA is no longer clustered in *rlf6* cells, we cannot rule out the alternative possibility that *RLF6* may be required only for the clustering of Rap1p antigen. Further studies using fluorescence *in situ* hybridization and telomeric DNA probes in *rlf6* mutant strains should shed light on this issue.

The inability to express heterologous genes in *Chlamydomonas* has been a technical hurdle to molecular studies of this organism. Much of the difficulty has been attributed to the high G+C content (65%) and unusual codon usage in *Chlamydomonas* genes. The codon usage of *GBP1* is similar to that of

Table 2. TPE in wild-type and *rlf6* strains

| Strain | Fraction* | |
|---|---|---|
| | Ura$^+$ | 5-FOA$^R$ |
| YJB917 (*RLF6*) | 1.1 (0.77–1.3) | $7.5 \times 10^{-2}$ (54–2.7) |
| YJB918 (*rlf6*) | 0.93 (0.50–1.2) | $3.5 \times 10^{-2}$ (2.3–5.7) |

Ura$^+$, uracil present; 5-FOA$^R$, 5-fluoroorotic acid resistant.
*Range in parentheses.

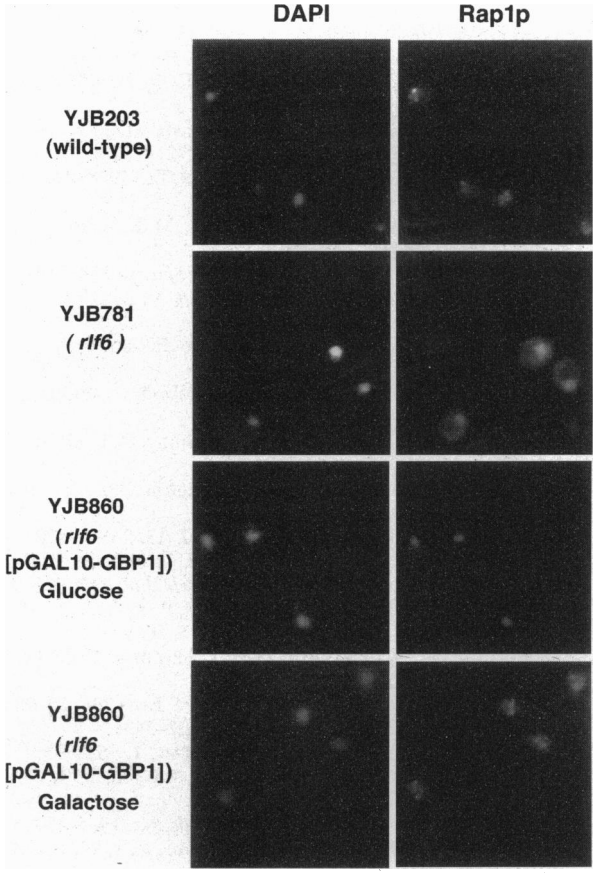


FIG. 3. Rlf6p is required for Rap1p localization and the expression of *GBP1* restores Rap1p localization in *rlf6* cells. Total DNA was detected with DAPI; Rap1p was detected with anti-Pep1 (7). YJB203, wild-type cells; YJB781, *rlf6::LEU2-B4*; YJB860 (YJB781 carrying pGAL10-GBP1). Similar diffuse, nuclear Rap1p staining was observed for YJB773, YJB776, YJB783 (yeast strains carrying other disruption alleles of *RLF6*), as well as for YJB868 [YJB781 carrying the vector plasmid pBM272 (52)] and YJB862 (YJB783 carrying pGAL10-GBP1) grown on glucose. Punctate Rap1p staining was also observed for YJB862 grown on galactose and for YJB894 and YJB895 grown on either galactose or glucose.

other *Chlamydomonas* genes (29). The complementation of *rlf6* mutations by *GBP1* expression clearly demonstrates that *Chlamydomonas* genes can be functionally expressed in *Saccharomyces*, in spite of the differences in codon usage. [*S. cerevisiae* genome is ≈61% A+T (59).] Our work suggests that yeast molecular genetic techniques, such as the two-hybrid system (60), should be useful for studying interactions between *Chlamydomonas* proteins.

A number of vertebrate hnRNPs bind telomeric G-strand DNA (25–27) and include RRM domains. The fact that Gbp1p can functionally restore Rap1p localization in *rlf6* mutants suggests that Gbp1p and Rlf6p are members of a conserved class of proteins that bind ss G-strand telomeric DNA and that are involved in the nuclear organization of telomeres and/or telomere-associated proteins. It will be of interest to determine whether any of the vertebrate hnRNPs are functionally related to the Gbp1p/Rlf6p class of telomere-binding proteins and whether, as in yeast, they play a role in the nuclear organization of telomeres or telomere-associated proteins.

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