Properties of the transcriptional enhancer in Saccharomyces cerevisiae telomeres

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ABSTRACT

Saccharomyces cerevisiae chromosomes end with the sequence C\(^{1-3}A\), commonly abbreviated as C\(^{1-3}A\). These sequences can function as upstream activators of transcription (UAS's) when placed in front of a CYC1-lacZ fusion gene. When C\(^{1-3}A\) sequences are placed between the GAL1,10 UAS and the CYC1-lacZ fusion, the C\(^{1-3}A\) UAS still functions and the amount of \(\beta\)-galactosidase produced in cells grown on glucose is as much or more than that for cells grown on either glycerol medium, or cells grown on glucose medium containing a plasmid with just the C\(^{1-3}A\) UAS. These data indicate that the UAS is immune from glucose repression from the upstream GAL1,10 UAS. Because C\(^{1-3}A\) sequences are bound \emph{in vitro} by the transcription factor RAP1, the UAS activity of yeast telomere sequences was compared with that of a similar UAS from the tightly regulated ribosomal protein gene RP39A, which also contains a RAP1 binding site. While transcription from the ribosomal protein gene UAS was responsive to cell density, the amount of transcription from the C\(^{1-3}A\) UAS was nearly the same at all cell densities tested. These data show that the transcriptional activation by C\(^{1-3}A\) sequences is not regulated by cell density.

INTRODUCTION

Telomeres are the physical ends of eukaryotic chromosomes. They protect internal sequences from exonucleolytic degradation and end-to-end fusion, and provide a solution to the dilemma of how to replicate completely a double-stranded DNA end (reviewed in [1]). These properties suggest that specific telomere binding proteins play a role in the replication and maintenance of telomeres. In the study reported here, it was our goal to devise a genetic screen for mutants in telomere-binding proteins. In the course of this work, we found that yeast telomere DNA sequences function as an upstream transcriptional activator (UAS) with some unusual properties.

It was shown several years ago that by placing a recognition site for the DNA binding protein \emph{lexA} between the GAL1,10 UAS and its TATA box and then supplying \emph{lexA} protein in trans, one could attenuate the transcriptional activation of the GAL1,10 UAS. It was also shown that placing the site at a number of positions between the UAS and TATA box would inhibit transcription [2]. Although the mechanism by which the \emph{lexA} protein interferes with transcription activation is still unclear (reviewed in [3]), this result suggested a general approach for assaying yeast DNA-binding proteins \emph{in vivo}. In place of the \emph{lexA} binding site, one can insert the sequence of interest and then ask for interference with GAL1,10-mediated transcription activation. One can then look for mutants that partially or completely remove this inhibition, some of which may be in the gene encoding the DNA-binding protein which caused the initial repression of transcription. We have conducted such an experiment with yeast telomeric DNA sequences (C\(^{1-3}A\) repeats) to search for proteins that interact with yeast telomeres. During the course of these experiments, we found that the yeast telomere sequence C\(^{1-3}A\) functions as a UAS (others have also reported that telomere sequences can act as a UAS [4]) and is unaffected by glucose repression from the upstream GAL1,10 UAS. This result is in marked contrast to a similar fusion made to the HIS3 UAS in which the HIS3 transcription was reduced about 80% on glucose by the upstream GAL1,10 UAS [5].

Because C\(^{1-3}A\) sequences are bound \emph{in vitro} by the transcription factor RAP1, the UAS activity of yeast telomere sequences was compared with that of a similar UAS from the tightly regulated ribosomal protein gene RP39A, which also contains a RAP1 binding site. The control of ribosomal protein gene transcription is linked to cell growth rate (reviewed in [6]). We tested the the activity of the C\(^{1-3}A\) UAS and the tripartite RP39A UAS at different cell densities. While transcription from the ribosomal protein gene UAS was responsive to cell density, the amount of transcription from the C\(^{1-3}A\) UAS was nearly the same at all cell densities tested.

MATERIALS AND METHODS

\emph{Yeasts and bacterial strains and methods.} All yeasts and bacterial media, yeast lithium acetate and bacterial transformation procedures have been previously described [7]. All experiments reported here were conducted in yeast strain KR14-86 (\(M\emph{ATa}\) ade2-I ade8-18 ura3-52 trp1\(\Delta\) leu2-3,112 his4) and KR9-14 (\(M\emph{ATa}\) ade2-1 ade8-18 ura3-52 trp1\(\Delta\) met2). All plasmid constructions were performed in \emph{E. coli} strain MC1000 (r\(^{-}\) m\(^{-}\) pyrF::Mu trp lac). Assays for \(\beta\)-galactosidase activity in yeast were performed as described by Guarente [8]. All values reported for \(\beta\)-galactosidase activity are the results of at least three independent cultures of the same transformant where each culture
Plasmid constructions. Plasmids were constructed using the methods described previously [7]. The plasmids YEpGZ, YEpGCAZ, YlpGZ, YlpCAZ, YEpGcaZ, YEpGgtZ, YlpCAZ, and YlpGtZ were individually cut with Stul (which cuts only once in these plasmids in the 3' end of the URA3 gene) and Xhol, treated with T4 DNA polymerase to render the 3' strands blunt-ended. These fragments were individually ligated to the 0.4 kb Stul-Smal restriction fragment from the URA3 gene (isolated from the YIp5 plasmid [10] and the ligation mix was used to transform the bacterial strain MC1000. In order to isolate those plasmids containing an intact URA3 gene, transformants were tested for their ability to grow on minimal media lacking uracil (the yeast URA3 gene was expressed from the Yip plasmids) by cutting with Stul to target the plasmids using the lithium acetate procedure. Yeast integrating plasmids (Yip versions) were constructed from pLGSD5 [9]. YEpGZ is the same plasmid as pLGSD5 and contains the 2μ origin of replication, the URA3 gene and a GAL1,10 UAS-CYC1 TATA region-lacZ fusion (fig. 1). This plasmid contains a single XhoI site between the GAL1,10 UAS and the CYC1 TATA region. YEpGZ was linearized with XhoI and a 125 bp SalI-XhoI fragment containing 81 bp of C1-A from YTCA-1X [7] was ligated into this site to generate YEpGCAZ. This plasmid contains the C1-A fragment in the orientation (reading the top strand 5' to 3') GAL1,10 UAS-XhoI-G1-T-SalI/XhoI-CYC1 TATA (see fig. 1). Both YEpGZ and YEpGCAZ were converted to yeast integrating plasmids (Yip versions) by cutting with HindIII, isolating the largest fragments and recircularizing by ligating at low DNA concentration. This procedure removes the 2μ origin of replication so that these plasmids cannot replicate autonomously in the cell.

YIpGZ and YlpGCAZ were used to construct, respectively, YIpZ and YlpCAZ. YIpGZ and YlpGCAZ were individually cut with Stul (which cuts only once in these plasmids in the URA3 gene) and XhoI, treated with T4 DNA polymerase to render the ends blunt-ended, and the largest fragments were isolated from an agarose gel. These fragments were individually ligated to the 0.4 kb Stul-Smal restriction fragment from the URA3 gene (isolated from the YIp5 plasmid [10]) and the ligation mix was used to transform the bacterial strain MC1000. In order to isolate those plasmids with an intact URA3 gene, transformants were tested for their ability to grow on minimal media lacking uracil (the yeast URA3 gene will complement the E. coli pyrF mutation [11]). These plasmids, called YlpZ and YlpCAZ, contain a precise deletion of the GAL1,10 UAS and a XhoI site at the 3' end of the URA3 gene. The plasmids YlpZ and YEpGZ were cut with XhoI and ligated to the C1-A oligonucleotides (sequence shown in fig. 1) to form YlpCAZ, YlpGtZ, YEpGcaZ and YEpGgtZ. YlpCAZ and YEpGcaZ are in the same orientation as YEpGCAZ, while the gt plasmids have the inserts in the reverse orientation. All plasmids were transformed into yeast using the lithium acetate procedure. Yeast integrating plasmids (Ylp plasmids) were first cut with Stul to target the plasmids to the URA3 gene. Yeast transformed with the Ylp plasmids were screened by Southern hybridization to isolate those strains that contained only one plasmid per genome.

The plasmid YlpRPG was derived from pMR672A (see [12]; kindly provided by J. Woolford). The same method that Rotenberg and Woolford used to convert pMR10 to pMR11 [12] was used to convert pMR672A to YlpRPG.

RESULTS

The Ylp plasmids contain no ARS and were integrated in single copy at the URA3 locus. The YEpl plasmids are extra chromosomal replicating vectors by virtue of the 2μ origin of replication. All plasmids contain the gene for β-galactosidase (lacZ) fused to the CYC1 TATA region (designated Z in the

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**Fig. 1.** The plasmids used in this study. The structure of the family of plasmids used in this study is diagramed. The large arrow is the 125 bp CA fragment and the small arrows are the 35 bp oligonucleotide. The letters (CA, ca or gt) indicate the insert's orientation (see Methods). Examples of integrating plasmid names are shown. The sequences of the B) 125 bp insert with 81 bp of C1-A and the 35 bp oligonucleotide are shown. The complementary strand to both is shown. The sequences which conform to RAPI binding sites as determined by Buchman et al. [4] are underlined. The 35 bp oligonucleotide contains no exact matches to this consensus and only one 12/13 match and is underlined with a dashed line. The nonconforming base is in italics. Both the 125 bp fragment containing 81 bp of C1-A and the 35 bp oligonucleotide are bound by a factor in crude yeast cell extracts prepared by the method of Berman et al. [13]. A) The RAPI consensus sequence is shown.
plasmid name). These constructs alone do not support detectable levels of β-galactosidase production in yeast, but require the addition of an upstream activator sequence, or UAS, to activate transcription. The addition of the 365 bp GAL1.10 UAS upstream of the CYC1 TATA region places the lacZ gene under GALA control. We have inserted short stretches of the yeast telomeric DNA sequence C\_3A into the XhoI site (designated by CA in the plasmid name) and placed these constructs into cells and assayed them for β-galactosidase activity (Table I). Cells were grown on glucose, glycerol or galactose in order to gauge the effect of the C\_3A sequences upon the GAL1.10 UAS under conditions of transcriptional repression (glucose medium), lack of repression and lack of transcriptional activation (glycerol medium) and transcriptional activation (galactose medium).

As had been previously reported, cells bearing plasmids containing only the GAL1.10 UAS and no C\_3A sequences produced no β-galactosidase on glucose and glycerol media and large amounts of enzyme when grown on galactose media (Table I A) [9]. Those cells bearing plasmids that contained the GAL1.10 UAS and a 125 bp insert with 81 bp of C\_3A sequences produced β-galactosidase on glucose and glycerol media, although the amount of enzyme produced was lower than that for cells grown on galactose medium. This observation is in contrast to a similar experiment with HIS3 where HIS3 gene expression was reduced ~80% on glucose media by an upstream GAL1.10 UAS.

Table I: β-galactosidase produced in yeast KR14-86 bearing the indicated plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>A. 81 bp C_3A Insert</th>
<th>B. 35 bp C_3A Insert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon source:</td>
<td>glucose</td>
<td>glycerol</td>
</tr>
<tr>
<td>YEpGZ</td>
<td>0.027</td>
<td>0.037</td>
</tr>
<tr>
<td>±</td>
<td>0.005</td>
<td>0.015</td>
</tr>
<tr>
<td>YIpGCAZ</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>±</td>
<td>0.15</td>
<td>0.26</td>
</tr>
<tr>
<td>YEpGCAZ (pLGSDS)</td>
<td>0.047</td>
<td>0.035</td>
</tr>
<tr>
<td>±</td>
<td>0.013</td>
<td>0.009</td>
</tr>
</tbody>
</table>

While this work was in progress, several groups reported the isolation, cloning and/or characterization of a protein, called, alternatively, RAPI, GRFI, TUF or TBA, that can bind to sequences in yeast telomeres [4, 13, 14, 15, 16]. All of these activities are believed to be the same protein, and will be referred to here as RAPI. The RAPI protein was originally isolated as a protein that can bind to the E-box of the yeast silent mating type cassettes [14, 15], and the consensus binding site has been defined as A/G C/A ACCCANNCA C/T C/T [4] which occurs 5' to many genes as well as at yeast telomeres. The consensus sequence from the yeast telomere is a high-affinity RAPI binding
A. YIpCAZ

Fig. 2. The RP39A UAS, but not the C₁₋₃A UAS, is regulated by cell density. Units of β-galactosidase activity (nmol ONPG hydrolyzed per min per 10⁷ cells) from different cultures are plotted against the cell density of the respective culture. A) Cells bearing YIpCAZ. B) Cells bearing YIpRPG. Each point represents the value for a single culture where each culture was assayed in triplicate. The standard deviation of each culture was 10% or less.

DISCUSSION

We have examined several properties of a UAS present in sequences from a cloned yeast telomere. While this work was in progress, several groups reported the isolation of an activity, referred to here as RAPI, that can bind to some of the combinations of C₂₋₃A(CG)₁₋₄ sequences present at yeast telomeres. These workers have shown that the RAPI binding sites from telomeres are high-affinity sites [13, 14], can function as a UAS in yeast [4] and are important for transcription in vitro [4]. We have extended these observations with our studies using oligonucleotides and cloned yeast telomere sequences to show that this UAS is immune from glucose-mediated repression from an upstream GALI.10 UAS (Table I) and that the C₁₋₃A UAS is regulated differently from the tripartite RP39A UAS with respect to cell density. One similarity between the RP39A UAS and the C₁₋₃A UAS is that ribosomal protein genes show a 2.5 to 4 fold increase in mRNA when shifted from a nonfermentable carbon source to glucose (see [6]). We found that plasmids containing the 35 bp C₁₋₃A oligonucleotide made up to 3 times as much β-galactosidase on glucose media as on glycerol (Table I B), suggesting an increased rate of transcription from the C₁₋₃A UAS on glucose medium compared to a nonfermentable carbon source. The lack of a similar effect in plasmids bearing the 125 bp insert may be due to the multiple RAPI binding sites in this fragment or the 44 bp of non-C₁₋₃A sequences interfering with these subtle differences in UAS activity. However, the RP39A UAS and C₁₋₃A UAS did not respond in the same way to changes in cell density. These observations indicate that regulation of ribosomal protein gene expression by cell density does not involve major changes in RAPI activity, and that the regulation of YIpRPG with respect to cell density may arise from interactions of other proteins with the HOMOL1 box or the T-rich region.

The observation that the C₁₋₃A UAS sequences used in this study are immune from glucose-mediated repression from the
upstream GAL1,10 UAS is surprising when compared to similar studies performed with the HIS3 gene. When the 365 bp GAL1,10 UAS was placed upstream of both HIS3 UAS sequences (the constitutive A11 UAS and the inducible GCN4 UAS) and inserted at the chromosomal HIS3 locus, HIS3 gene expression was always partially repressed to about 20% of its control value when glucose was the carbon source [5]. These data suggest that RAP1 protein mediated transcription may be specifically immune from glucose repression. The binding of RAP1 protein nearer the TATA box may specifically abrogate the effects of glucose repression-mediating proteins that bind at the upstream GAL1,10 UAS.

Buchman et al. have suggested that RAP1 represents a general transcription factor in yeast similar to SP1, CCAAT box binding factor, and octamer binding factor in mammalian cells [4]. However, this proposal does not suggest how RAP1 protein acts at telomeres. Gasser and co-workers have suggested that RAP1 may attach yeast telomeres to the nuclear scaffold [18]. Thus, RAP1 may possess a telomere-specific function distinct from transcription activation. Any hypothesis for RAP1 action at telomeres must address the fact that yeast cells can support 200–800 new telomeres per cell and a 10–25 fold increase in telomeric C3A sequence with no effect upon cell growth rate or chromosome stability ([7] and Runge, Wellinger and Zakian, in preparation). The only known phenotype is an increase in the length of terminal C3-A tracts ([7]). Assuming three RAP1 binding sites per 80 bp (as in the cloned yeast telomere sequences in YIpCAZ), this increase in C3-A sequence exceeds the estimated total number of RAP1 protein molecules per cell (~ 4000 molecules per cell [4], vs. 6000–16000 sites [7]). Since RAP1 appears to bind to telomeres in vivo (M. Conrad, J. Wright, and V. Zakian, in preparation), its presence at telomeres may be dispensable for telomere function with regard to replication and end stabilization.

Does the transcriptional activation properties of the C3-A UAS serve a function in telomere replication? In the ciliates Tetrahymena, Oxytricha, and Euplotes, an enzymatic activity has been isolated that adds telomeric DNA repeats to DNA termini in the absence of a complementary DNA strand [19, 20, 21]. This activity, known as telomerase, contains an RNA that is required for function. It has been proposed that this RNA provides the template for the addition of telomeric DNA sequences [19]. In the case of the Tetrahymena enzyme, the RNA has been cloned and sequenced. It is most likely an RNA polymerase III transcript and contains 159 nucleotides, 9 of which can serve as a template for Tetrahymena telomere synthesis [19]. It is conceivable that RAP1 protein binding in telomeric DNA sequences serves to promote the transcription of C3-A RNA's that are used in a similar enzyme in S. cerevisiae. However, transcription promoted within the telomere would yield a long RNA that contains mostly C3-A sequences, quite different from the RNA in Tetrahymena (e.g. a small RNA with only a 9 bp stretch of telomere sequence). Thus, if yeast contains a telomerase with a short RNA template similar to that in Tetrahymena, it is unlikely to be transcribed from the terminal C3-A repeats.

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