INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS EN BIOLOGÍA MOLECULAR

ANÁLISIS DE LA INTERACCIÓN ENTRE LAS PROTEÍNAS ABF1 Y RAP1 DE *CANDIDA GLABRATA* CON LOS ELEMENTOS DE REGULACIÓN EN *CIS* DEL TELÓMERO E DERECHO

Tesis que presenta

LEONARDO CASTANEDO IBARRA

Para obtener el grado de

Maestro en Ciencias en Biología Molecular

Director de la Tesis:

Dra. Irene Castaño Navarro

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Constancia de aprobación de la tesis

La tesis ***"Análisis de la interacción entre las proteínas Abf1 y Rap1 de Candida glabrata con los elementos de regulación en cis del telómero E derecho"*** presentada para obtener el Grado de Maestro en Ciencias en Biología Molecular fue elaborada por **Leonardo Castanedo Ibarra** y aprobada el **dos de septiembre del dos mil quince** por los suscritos, designados por el Colegio de Profesores de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

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sobre la Tesis intitulada:

Análisis de la interacción entre las proteínas Abf1 y Rap1 de Candida glabrata con los elementos de regulación en cis del telómero E derecho

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Dedicatorias

…A MI MADRE ES A QUIEN ESPECIALMENTE LE DEDICO ESTE TRABAJO. LE AGRADEZCO POR SER UN EJEMPLO PARA MÍ Y PARA EL MUNDO, POR ENSEÑARME A PERSEVERAR POR MIS IDEALES Y POR SU VALENTÍA Y FUERZA INAGOTABLES, PARA LUCHAR POR SU VIDA Y LA DE SUS HIJOS

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Abreviaturas (por sus singlas en inglés)

EPA Epithelial Adhesin

E-Rt Right telomere, chromosome E

I-Rt Right telomere, chromosome I

T-loop Telomere Loop

Abf1 ARS-Binding Factor 1

Rap1 Repressor-Activator Protein 1

Sir Silent Information Regulator Proteins

c-Myc MyeloCytomatosis proto-oncogen protein

3D Three-Dimensional space

CREs *Cis*-Regulatory Elements

GTFs General Transcription Factors

GSTFs Gene-Specific Transcription Factors

Kb Kilobases

NE Negative element

C-terminal Carboxyl terminal

ChIP Chromatin Immunoprecipitation

SC Synthetic complete

NH_2SO_4 Ammonium sulfate

CAA Casamino Acids

5-FOA 5-Fluoroorotic acid

YPD Yeast extract-Peptone-Dextrose

OD Optical Density

LiAc Lithium acetate

SS Salmon sperm DNA

PEG Polietilenglicol

NAT Nourseothricin resistanece marker

Bp Base pair

Nat^{R} Nourseothricin-resistance colonies

UTR Untranslated region

ARS Autonomously replicating sequence

ORF Open reading frame

FRT Flp Recombination Targets

CEN Centromere

ChIP Chromatin immunoprecipitation

Aa Aminoacids

Bp Baise pair

Kb Kilobase

kDa Kilodalton

Abstract

"Analysis of the interaction between the Abf1 and Rap1 proteins of *Candida glabrata* with the *cis*-acting regulatory elements of the E-R telomere"

Regulation of gene expression in eukaryotes is governed by the binding of transcription factors to *cis*-acting elements in the genome. In the pathogenic yeast, *Candida glabrata*, an example of this regulation is the *EPA* (Epithelial Adhesin) gene family, which encodes cell surface proteins that mediate adhesion of the yeast to epithelial host cells. In the strain BG2, *EPA1* is the only gene of this family that is expressed *in vitro*, whereas the remaining 22 *EPA* paralogues are transcriptionally repressed. *EPA1* forms a cluster with *EPA2* and *EPA3* close to the right telomere of the Chromosome E (E-Rt). Contrary to the widespread silencing effect over the subtelomeric regions observed in other *C. glabrata* telomeres, the E-Rt exhibits a combination of regional and local silencing of the *EPA* genes.

This effect could be due to repression at a distance, by telomere looping (T-loop), where the DNA-binding proteins Abf1 and Rap1 participate through the recruitment of the Sir proteins, which are completely necessary for the silencing at subtelomeric regions in *C. glabrata*. We tagged the Abf1 and Rap1 proteins with c-Myc and FLAG epitopes to study their function at *Candida glabrata* subtelomeric regions. We found that the tags can interfere with the normal silencing activity of the proteins and in the process, we determined that Abf1 and Rap1 participate in the regulation of the expression of *EPA* genes at the E-R and I-R telomeres, possibly through telomere loop formation.

Key words: silencing, telomeres, t-loops, regulation, yeast.

Resumen

"Análisis de la interacción entre las proteínas Abf1 y Rap1 de *Candida glabrata* con los elementos de regulación en *cis* del telómero E-R"

La regulación de la expresión génica está gobernada por la unión de los factores de transcripción a los elementos de regulación en *cis* del genoma eucariota. En el patógeno *Candida glabrata*, un ejemplo de este tipo de regulación se encuentra representado por la familia de genes *EPA* (adhesina epitelial, por su siglas en inglés), que codifican para proteínas de la pared celular, las cuales, median la adhesión de la levadura a las células epiteliales del hospedero. En la cepa BG2, *EPA1* es el único gen de la familia *EPA* que se expresa *in vitro,* mientras los 22 parálogos *EPA* permanecen transcripcionalmente reprimidos o silenciados. *EPA1* está codificado en la región subtelomérica del cromosoma E izquierdo (tE-R), adyacente a *EPA2* y *EPA3*.

Las regiones subteloméricas de *C. glabrata* presentan un silenciamiento regional, no obstante, el tE-R manifiesta una combinación de silenciamiento regional y local sobre los genes *EPA* localizados allí. Este efecto podría deberse a la formación de bucles teloméricos (T-loop) al extremo del cromosoma, posiblemente mediado por las proteínas de unión al ADN, Abf1 y Rap1, mediante el reclutamiento de las proteínas Sir, las cuales son necesarias para el silenciamiento subtelomérico en *C. glabrata*. Para estudiar la función de éstas proteínas en el silenciamiento subtelomérico de *C. glabrata*, las etiquetamos con los epítopos c-Myc y FLAG y encontramos que las etiquetas pueden interferir con la función nativa de las proteínas. Adicionalmente, determinamos que Abf1 y Rap1 participan en la regulación de la expresión de los genes *EPA* de los telómeros E-R e I-R, posiblemente a través de la formación de T-loops.

Palabras clave: silenciamiento, telómeros, t-loop, regulación, levadura.

1. Abstract

"Analysis of the interaction between the Abf1 and Rap1 proteins of *Candida glabrata* with the *cis*-acting regulatory elements of the E-R telomere"

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Regulation of gene expression in eukaryotes is governed by the binding of transcription factors to *cis*-acting elements in the genome. In the pathogenic yeast, *Candida glabrata*, an example of this regulation is the *EPA* (Epithelial Adhesin) gene family, which encodes cell surface proteins that mediate adhesion of the yeast to epithelial host cells. In the strain BG2, *EPA1* is the only gene of this family that is expressed *in vitro*, whereas the remaining 22 *EPA* paralogues are transcriptionally repressed.

EPA1 forms a cluster with *EPA2* and *EPA3* close to the right telomere of the Chromosome E (E-Rt). Contrary to the widespread silencing effect over the subtelomeric regions observed in other *C. glabrata* telomeres, the E-Rt exhibits a combination of regional and local silencing of the *EPA* genes.

This effect could be due to repression at a distance, by telomere looping (T-loop), where the DNA-binding proteins Abf1 and Rap1 participate through the recruitment of the Sir proteins, which are completely necessary for the silencing at subtelomeric regions in *C. glabrata*. We tagged the Abf1 and Rap1 proteins with c-Myc and FLAG epitopes to study their function at *Candida glabrata* subtelomeric

regions. We found that the tags can interfere with the normal silencing activity of the proteins and in the process, we determined that Abf1 and Rap1 participate in the regulation of the expression of *EPA* genes at the E-R and I-R telomeres, possibly through telomere loop formation.

2. Introduction

During its evolution, the eukaryotic genome has reached a high degree of complexity and has acquired different levels of organization with multiple layers of regulation. The eukaryotic DNA is arranged in a set of individual chromosomes that consist of linear DNA associated with proteins that form the chromatin. The chromatin structure is organized at three different levels: 1) the first is the nucleotide sequence of the chromosomes, which comprises the coding regions, the regulatory sequences and intrinsic elements of the DNA that influence the 3D folding of chromatin structure; 2) the second level of chromatin organization is defined by the specific composition of histones proteins, and their post-translational modifications; and 3) the third level is the 3D disposition of the genome in the nucleus (Turner 2002; van Driel *et al.* 2003). The conformation of the eukaryotic genome in three different hierarchical levels allows the precise regulation of stage-specific genes and proper gene expression according to external stimuli the cell receives (Jaenisch and Bird 2003).

The sequence level of genome organization, which represents the regulation of individual or a small group of genes, is the best studied (van Driel *et al.* 2003). In the regulation at the sequence level, the intrinsic DNA elements are defined as *cis*-regulatory elements (CREs), that are the modules where the general

transcription factors (GTFs), sequence-specific DNA binding proteins (gene-specific transcription factors [GSTFs]) and other protein complexes directly bind and/or interact. Some CREs, as promoters and positive and negative elements, allow the activation or the repression of a gene in a specific, promoter-dependent manner, which depends on the RNA polymerase II, GTFs, co-activator and co-repressor complexes (Lowings *et al.* 1992; Ohi *et al.* 1994; Rusche *et al.* 2003; Gallegos-García *et al.* 2012). On the other hand CREs, such as silencers, protosilencers, and enhancers, act in concert with DNA-binding proteins, histone modifyng enzymes and histone binding proteins, to facilitate the formation of a specialized chromatin structure, that blocks or activates the expression of the genes in a domain or region, independent of the promoter or the gene itself (Boscheron *et al.* 1996; Blackwood and Kadonaga 1998; Lebrun *et al.* 2001; Juárez-Reyes *et al.* 2012).

Promoter-independent repression, is known as gene silencing (Moazed 2001; Rusche *et al.* 2003), and the telomeres of some yeast, such as the human pathogen *Candida glabrata*, exhibit subtelomeric silencing, where the telomere-binding protein Rap1 and the chromatin-modifiers Sir proteins are necessary for full repression of these regions (Castaño *et al.* 2005; De Las Peñas *et al.* 2003). Contrary to the widespread silencing effect throughout the subtelomeric regions of the *C. glabrata* telomeres, the E-R telomere (E-Rt), where *EPA1-EPA3* are encoded, exhibits a combination of regional and local silencing (De Las Peñas *et al.* 2003; Rosas-Hernández *et al.* 2008). The discontinuous silencing effect in the E-Rt, could be the result of the interaction at a distance, between the telomere and a CRE ≥20 kb (kilobases) away from it toward the centromere. We have proposed

that the discontinuous silencing was the result of the interaction between two CREs characterized at the E-Rt, a negative element (NE) and a protosilencer (Sil2126) (Gallegos-García *et al.* 2012; Juárez-Reyes *et al.* 2012), and since Sil2126 has two putative binding sites for silencing proteins, one for Rap1 and the other for Abf1, and genetic evidence suggests that yKu70 and yKu80 proteins interact with the NE, it was proposed that possibly these two CREs could interact through protein-protein interactions, to repress the expression of *EPA1*, *EPA2*, and *EPA3* in a regional manner.

In this work, we generated C-terminal tagged (using c-Myc and FLAG epitopes) versions of the Abf1 and Rap1 proteins to determine if they bind to their respective CREs using Chromatin Immunoprecipitation assays (ChIP assays). We show, that although the tagged Rap1 versions are functional for silencing at telomeres of *C. glabrata*, additional functions, such as the activation of the gene transcription (e.g. ribosomal and glycolytic genes), might be compromised by the epitopes. The function of Abf1 was also altered by the tags. Surprinsigly we found that modification of the C-terminal domain by either c-Myc or FLAG or alternatively disrupting it by a deletion of the last 43 residues, which corresponds to a conserved domain of Abf1, diminishes silencing at E-Rt and the I-Rt. This is the first evidence that suggests that Abf1 participates in subtelomeric silencing in *C. glabrata*.

3. Material and Methods

3.1. Strains, plasmids, and primers. All strains, plasmids and oligonucleotides used are listed in Table S1, Table S2, and Table S3 respectively.

3.2. Media. Yeast were grown in standard yeast media as described previously with 2% agar added for plates (Sherman *et al.* 1986). Synthetic complete (SC) contains 1.7 g/liter yeast nutrient base (without NH_2SO_4 and amino acids), 5 g/liter NH_2SO_4 and supplemented with 0.6% casamino acids (CAA) and 2% glucose. When needed, SC was supplemented with 25 mg of uracil/liter. To prepare 5-fluoroorotic acid (5-FOA; Toronto Research Chemicals) media, 0.9 g of 5-FOA and 25 mg of uracil/liter were added to the SC. Yeast extract-peptone-dextrose (YPD) medium contains 10 g/liter yeast extract, 20 g/liter peptone, and is supplemented with 2% glucose. When required, YPD plates were supplemented with Nourseothricin (Invitrogen^{TM}) at 100 mg/ml.

Bacteria were grown in LB medium as described previously (Ausubel *et al.* 2001). LB medium contained 5 g/liter yeast extract, 10 g/liter tryptone, 5 g/liter NaCl. All plasmid constructs were introduced into strain DH10 by electroporation, and 100 µg/ml carbenicillin (Invitrogen^{TM}) was added to select for plasmids. For plates, 1.5% agar was used.

3.3. Reporter *URA3* gene expression assays (5-FOA sensitivity assays). The level of silencing of the *URA3* gene inserted at different positions throughout the telomeres was assessed using a plate growth assay as described previously (De Las Penas *et al.* 2003; Castano *et al.* 2005). Strains containing different *URA3* insertions were grown in YPD for 48 h to stationary phase. The cultures were adjusted to an optical density (OD_{600}) of 1 with sterile water, and 10-fold serial dilutions were made in 96-well plates. A total of 5 µl of each dilution was spotted

onto YPD, SC lacking uracil, and SC + 5-FOA plates, with the replica-plating tool (NUNC™). The plates were incubated for 48 h at 30 °C, and then photographed.

3.4. Yeast transformation. Yeast transformations with digested or supercoiled plasmids were performed as previously described using the LiAc/SS carrier DNA/PEG method (Castaño *et al.* 2003; Gietz and Schiestl 2007).

3.5. Growth assays in liquid and solid media. Cells were grown to stationary phase for 48 h hours in YPD. For the liquid media experiments, the cultures were adjusted to an OD_{600} of 0.03 and 300 µL dispensed in a 100-well plate. Growth was automatically recorded using Bioscreen C analyser at 30 °C (Thermic Labsystems Oy, Finland) as previously described with constant shaking and OD measurements taken every 30 min during a period of 48 h (Warringer and Blomberg 2003). The doubling time of each strain was calculated as described elsewhere (Olsen *et al.* 2010).

For the solid media experiments, stationary phase cultures were adjusted to an OD_{600} of 1 with sterile water, and 10-fold serial dilutions were made in 96-well plates. A total of 5 µl of each dilution was spotted onto YPD, then incubated at four different temperatures (30 °C, 37 °C, 45 °C and 47 °C), and photographed every 24 h during three days.

3.6. Construction of *C. glabrata* strains with c-Myc and FLAG tagged Abf1 and Rap1 integrations. We developed a strategy for translational C-terminal epitope-tagging of Abf1 and Rap1 proteins, which are the putative interactors of the

CREs at the E-Rt. The tagging vectors, pCI16-18 for the *ABF1* gene and pCI24-26 for the *RAP1* gene, were constructed as starting plasmids on integrative vectors pYC46, containing three FLAG epitope repetitions (3xFLAG) and pYC50 containing thirteen c-Myc repetitions (13xc-Myc) (Yáñez-Carrillo *et al.* 2015). Each construction carried, c-Myc or FLAG epitopes, a resistance marker to Nourseothricin (NAT) flanked by 34 bp FRT sites (Flp1 recombinase recognition sites). The PCR-amplified products of the C-terminal *ABF1* or *RAP1* genes from the *C. glabrata* (BG2 strain) were cloned 5' to the tag. The 3' untranslated region (3' UTR) of each gene was cloned after the second FRT site. The C-terminal fragment and 3'UTR were used as homology regions to integrate the constructions via homologous recombination in the *C. glabrata* genome (Fig. 1). Integration was achieved by digesting the corresponding vectors containing the *ABF1* constructs with *Bsr*GI/*Sal*I; and *Bsg*I/*Sal*I for the plasmids containing the *RAP1* modules. The linear fragments were used for transformation into two BG2 derived strains (*ura3*Δ and *URA3*+) and Nat^R colonies were selected on YPD plates supplemented with NAT. Four independent transformants were selected and checked for correct integration using PCR with specific primers (Fig. 1A).

3.7. Construction of the strains with the complementing vectors containing *ABF1* and *RAP1* genes. In parallel we constructed complementing vectors containing *ABF1* (pCI12) and *RAP1* (pCI28) with their own promoter and 3'UTR regions. These vectors will allow us to gain more insight into the function of Abf1 and Rap1 as silencing proteins in *C. glabrata*. The pCI12 and pCI28 vectors will allow us to complement a strain with deletions in both genes in case they are

essential. These plasmids contain the Open Reading Frame (ORF) of *ABF1* and *RAP1*, as well as a *URA3* selectable marker, an Autonomously Replicating Sequence (ARS) and a *C. glabrata* centromere (CEN). The 3.4 kb *CgABF1* gene (including the ORF and the 5' and 3' UTR of *ABF1*) was amplified using the primers #1589 and #1590 (see Appendixes, Table S3). A 3 kb PCR product corresponding to the *CgRAP1* gene (5'UTR+ORF+3'UTR) was amplified using the primers #1611 and #1612 (Table S1). These constructs were transformed as supercoiled plasmids into the BG2 strain, generating two independently BG14 derived strains (*ura3*Δ). Transformants were selected on SC media lacking uracil.

3.8. Construction of the *C. glabrata* strains with the truncated *abf1-43* allele. We aligned the sequence of the *Sc*Abf1 to the *Cg*Abf1 sequence to establish the boundaries of the CS2 silencing domain (CS2d), described elsewhere (Miyake *et al.* 2002). We found that this domain is highly conserved (87% identity and 96% of similarity), and used this information to generate a truncated allele *abf1-43*, which lacks the last 43 aa corresponding to the CS2d. The plasmid was constructed using the primers #1559 and #1880 (Table S3), as described above, using and integrative vector pYC44 with a *NAT* resistance gene and transformed in *C. glabrata* as a linearized plasmid.

3.9. Immunoblot detection of c-Myc and FLAG tagged Abf1 and Rap1 proteins. Logarithmic-phase yeast cells were grown at 30°C for seven duplications in YPD broth. These cultures were inoculated so that after 7 duplications, the OD_{600} of the cultures was 1.0. Cells were treated as previously described with a

few modifications (Orta-Zavalza *et al.* 2013). Cell were lysed in the FastPrep-24 homogenizer Instrument (MP Biomedicals®), in 100 µl of zirconia beads (Biospec®), by giving three pulses of 60 s at 6.0 m/s. Cells were placed on ice for 5 min between each pulse. The supernatant was transferred to a clean tube and the protein content was determined by Bradford assay (Fermentas®). Protein from cell extracts were separated by 10% polyacrylamide SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (PVDF, BIO-RAD®). The membranes were incubated with anti-c-Myc (Milipore®) or anti-FLAG (Sigma®) primary antibody solutions, and after 1.5 h and four washing steps with TTBS-Milk buffer (100 mM Tris-HCl, p.H. 7.5, 2.5 M NaCl, 0.05% v/v Tween 20 and 0.4% v/v Svelty Skim milk Nestlé®), the membranes were incubated with the Goat anti mouse IgG secondary antibody (Amersham®). The detection was made with the luminol/H_2O_2/piodophenol solution (Thermo Scientific®).

3.10. Scan for Abf1, Rap1 and ORC motifs. Since no motif databases, or consensus DNA binding sequences for either of the proteins of interest has been reported in *C. glabrata*, we used the motifs of *S. cerevisiae*, deposited at the database JASPAR (Sandelin *et al.* 2004). *Cg*Rap1 and *Sc*Rap1 has an overall identity in its double DNA-binding MYB domain (MYBd) of 75%, including the linker region; *Cg*Abf1p and *Sc*Abf1p have an 90% and 78% of identity in their Zinc finger domain and their specific DNA binding domain respectively.

We found that the DNA binding domains are highly conserved, we searched for putative DNA sites for both proteins. We used the MAST (E-value ≤10) and the FIMO (for *p*-value 1E-4) tool within the MEME package, which combine a set of

motifs to provide the best combined match, to scan the subtelomeric sequences of the E-R and I-R telomeres (Bailey *et al.* 2009). The motifs for Abf1 at the I-Rt couldn't be detected by MAST, in this case, we scanned for individual matches using FIMO (Bailey *et al.* 2009).

4. Results

4.1. Construction of epitope tagging and complementing *ABF1* and *RAP1* vectors. In order to determine whether the Abf1 and Rap1 proteins bind directly to the protosilencer Sil2126 or other subtelomeric CREs, we first developed a translational C-terminal epitope-tagging strategy of the proteins of interest with the c-Myc and FLAG epitopes, in order to use them in ChIP assays (Fig. 1A). Tagging of endogenous genes with specific epitopes is a widely used approach to detect the *in vivo* binding of TFs to specific CREs (Terpe 2002). The epitope specificity and antibody reactivity are parameters that enable the detection of protein complexes using ChIP assays, for that reason we decided to use two different epitopes and also because first, it is difficult to a priori choose an optimal affinity tag for a protein of interest, which depends in part on the protein itself (Terpe 2003; Landt et al. 2012); and second, the use of two epitopes will allow us to have strains with two differentially tagged proteins and therefore we can test whether Abf1 and Rap1 proteins bind to the same DNA sites and whether they form a complex. We generated a total of 4 integrative constructs containing *ABF1-c-Myc*, *ABF1-FLAG*, *RAP1-c-Myc*, *RAP1-FLAG* (See Tables S2 & S3). Following the scheme outlined in Fig. 1, we transformed *C. glabrata* with linearized plasmids and obtained the

corresponding transformants (C, G and T, Fig. 1A), with the integration of *ABF1* fused to c-Myc and FLAG tags independently and two transformants were obtained with each tag. We also obtained *RAP1-c-Myc* and *RAP1-FLAG* gene fusions using the same scheme shown in Fig. 1.

Deletion of *ABF1* in *S. cerevisiae* is lethal, but a deletion of its C-terminal conserved CS2d is viable (Fig. S1). Yet, the deletion of the CS2 affects gene activation, gene silencing and DNA replication in *S. cerevisiae* (Miyake *et al.* 2003). We made a deletion of the CS2 domain (deletion of the last 43 aa) in the Abf1 protein of *CgABF1* (Fig. S1) and the truncated allele (*abf1-43*), was transformed in *C. glabrata* as described above (see methods). As in *S. cerevisiae* the deletion of the CS2 in the *CgABF1* gene is viable but affects the capacity of *C. glabrata* to grow at high temperatures (Fig 3, compare the parental with the *abf1-43* strain). Although the temperature sensitivity observed when *S. cerevisiae* carries the truncated *abf1-43* allele at 37 ° C (Miyake *et al.* 2003) is not observed in the *C. glabrata abf1-43* strain, this could be a consequence of *C. glabrata* being a pathogen, thus *C. glabrata* shows more resistance to higher temperatures (47 °C) than the non-pathogenic yeast *S. cerevisiae* (Fig 3, see the parental strain at 45 °C and 47 °C).

4.2. Immunoblot detection. To confirm the presence of the tagged Abf1 and Rap1 proteins in the respective strains and to test if the antibody can successfully pull down the fusion proteins, western blot experiments were conducted. Fig. 2, shows the PVDF membranes revealed with luminol solution (see methods). Panels A and

B, correspond to the PVDF membranes incubated with the primary anti-c-Myc (αc-Myc) and anti-FLAG (αFLAG) antibodies respectively. In panel A, the positive control corresponds to the *Cg*Hst1-c-Myc, and the positive control, *Cg*Sum1-FLAG is shown in Panel B. Untagged protein extract was used as a negative control. Rap1 was immunoprecipitated efficiently with either c-Myc (lane 5) or FLAG epitopes (lanes 6 and 7). The detected bands correspond to the expected molecular weight: 94 kDa of Rap1-13xc-Myc, and 82 kDa of Rap1-3xFLAG (Fig. 2, marked with orange arrows, compare with controls). We could not detect Abf1-c-Myc or Abf1-FLAG epitopes, which are expected to have 69 kDa and 57 kDa respectively (Fig. 2, indicated with red arrows). Instead inespecific signals are detected in the 65 kDa and 25 kDa molecular weight region (Fig. 2B, marked with black arrows). The 70 kDa bands don't correspond to Abf1-FLAG with the expected 57 kDa weight. It could be general background, which is also observed, in the 25 kDa region of Panel B. According to these results the Rap1 proteins are present in the cell extracts and can be efficiently immunodetected using the c-Myc and/or FLAG epitopes. This is necessary to perform the ChIP assays, but first the functionality of the tagged proteins should be tested, in order to obtain reliable results in the consecutive experiments.

4.3. Growth assays in liquid and solid media. Defects in the functions of Abf1 and Rap1 proteins, have negative effects in the growth of *S. cerevisiae*, for example C-terminal truncations in Rap1, provokes lower growth rates compared with the parental strain, as a consequence of a cell cycle arrest (Kyrion *et al.* 1992); C-terminal truncations of *ABF1*, causes the cells to show temperature-

senstitive growth, due to a malfunction of chromosomal DNA replication (Miyake *et al.* 2002). In *C. glabrata*, the C-terminal conserved sites in Abf1 and Rap1, are also present, and have an identity of 87% and 64% respectively to the *S. cerevisiae* orthologs (Fig. S1). If the functions mediated by the C-terminal domains of Abf1 and Rap1 in *S. cerevisiae* are conserved in *C. glabrata*, then a malfunction of the tagged proteins could be reflected in growth assays in the *C. glabrata* strains. First, we tested the ability of the strains carrying the tagged proteins, to grow in liquid media, we calculated the doubling times of the parental and two independent transformants of each epitope, the results are shown In Table 1. The transformants have very similar duplication times at 30 °C (see methods), and significant differences were not observed between them and the parental strain. To further examine the phenotype of strains carrying the C-terminal fusions of Abf1 and Rap1 in *C. glabrata*, we assayed the growth in solid rich media at different temperatures, in these assays we used a temperature sensitive strain with a deletion of *HDF1* (*hdf1*Δ) gene, as negative control (Rosas-Hernández *et al.* 2008) and also, a C-terminal truncated allele of *RAP1* (*rap1-21*) that has a deletion of the last 28 aa (De Las Peñas *et al.* 2003) and the C-terminal deletion allele *abf1-43* were used. Fig. 3 shows the results of this experiment. The most marked effects in the growth on solid media of the tagged strains are seen at 47 °C, the wild-type strain at this temperature grows poorly (compare with the cells grown at 45 °C, 37 °C and 30 °C), and as expected, the *hdf1*Δ mutant is sensitive at 45 °C and 47 °C. Surprisingly, the *abf1-43* strain, is sensitive at 47 °C, while the parental strains at least the first two dilutions grow. It is important to note that, the tagged *Cg*Abf1 versions, like the *abf1-43* allele, are more sensitive at 47 °C than the wild-type

strain, suggesting that the function of Abf1 is affected by the tags and by the CS2d truncation in *C. glabrata*. The *rap1-21* mutant has growth defects at 47 °C, unexpectedly, the strains carrying the Rap1 tagged versions grow even more poorly than the *rap1-21* mutant at this elevated temperature.

4.4. Reporter *URA3* gene expression assays (5-FOA sensitivity assays). The C-terminal, RCT domain (RCTd) of Rap1 is essential for the Sir proteins recruitment at subtelomeric regions of *C. glabrata* (De las Peñas *et al.* 2003). Rap1 binds to the telomeric repeats of the chromosomes, and recruits the Sir complex, which is enriched at discrete regions of the telomere, thus creating zones of repressive chromatin (Ellahi *et al.* 2015). A disruption in the RCTd affects the repressive-state of *EPA* genes at E-Rt and this effect can be measured by the expression of the *URA3* reporter gene inserted at different locations in this telomere, importantly, the results inferred by *URA3* are consistent with alternative experiments and *in vivo* assays in different studies (De Las Peñas *et al.* 2003; Castano *et al.* 2005; Domergue *et al.* 2005; Rosas-Hernández *et al.* 2008; Gallegos-García *et al.* 2012). When *URA3* is transcriptionally active, the Ura3 protein converts the 5-FOA reagent added to the media into a toxic compound, therefore, cells expressing *URA3* are unable to grow. We tested the ability of our tagged Rap1 versions to silence a reporter *URA3* gene at different positions in E-Rt, using 5-FOA assays. As shown in Fig.4, both tagged Rap1 fusions mediate silencing of the reporter placed between *EPA2* and *EPA3*, compared with the allele

rap1-21, which lacks the entire RCTd and does not mediate silencing at this position (Fig. 4).

The function of Abf1 in *C. glabrata* is unknown, but the CS2d, which in *S. cerevsiae* mediates the silencing functions of the protein, is conserved (Fig. S1), additionally it is possible that Abf1 contributes to the silencing of the *EPA* genes, due to the presence of consensus binding sites for both, Abf1 and Rap1 in the E-Rt (Fig. S3). We measured silencing of the *URA3* reporter gene inserted at two different positions mediated by the tagged and the truncated Abf1 proteins (Insertion 2 and 3 of Fig. 5). Fig. 5 shows that the *URA3* reporter gene inserted between *EPA3* and the telomere, where the Sil2126 element is located, is efficiently silenced in the strain carrying the Abf1 fusions and the *abf1-43* allele (insertion 3 of Fig. 5). The silencing of the reporter gene inserted between *EPA2* and *EPA3*, in the tagged and truncated Abf1 proteins remains the same as the parental too (Fig. 5). However, the small amount of silencing of the chromatin region adjacent to *EPA1*, is completely lost, and this effect is consistent in the tagged Abf1 versions and when *C. glabrata* carries the defective *abf1-43* protein (Fig. 5 insertion 1) (Fig. 5). The effect of loss of silencing as a consequence of the tagging of Abf1 protein made us consider that the c-Myc and FLAG tags may affect the function of the Abf1 protein, analogously to the phenotype showed by a CS2d-disrupted *S. cerevisiae* strain (*abf1-43*) and also, that Abf1 could be an additional factor for the recruitment of the Sir proteins at subtelomeric regions in *C. glabrata*. To test this, we introduced the *abf1-43* allele in a *rap1-21* strain, expecting that the residual silencing observed at E-Rt between *EPA2* and EPA3 when *rap1-21* is present would disappear in the double mutant *rap1-21*/*abf1-43*. However, the

residual silencing is still present in the double mutant (Fig. 5, insertion 2, lane rap1-21/abf1-43). In an independent subtelomeric region, in the right telomere of chromosome I, the double mutant, *rap1*-21/abf1-*43* displays a reduced silencing between *EPA4* and *EPA5*, compared to the single *rap1-21* mutant (Fig. 5C and D).

5. Discussion

5.1. Epitope-tagging of Abf1 and Rap1 proteins.

The expression of the subtelomeric *EPA* genes in the BG2 *C. glabrata* strain depends on several layers of regulation, where CREs and specific proteins take part. One layer of regulation is composed of DNA binding proteins, such as Rap1, which recruits the Sir complex to subtelomeric regions, and another layer depends on specific proteins, their binding to the CREs and, the chromosomal context where the CREs are located.

We hypothesize that the proteins, Abf1 and Rap1 are involved in the silencing of the *EPA* genes located in the subtelomeric region of the E-Rt, through the protosilencer Sil2126 (Juárez-Reyes *et al.* 2012). To test this hypothesis, we first develop a translational C-terminal epitope-tagging strategy of Abf1 and Rap1 with the c-Myc and FLAG epitopes, to simultaneously use the recombinant fusion proteins in ChIP assays. FLAG and c-Myc were placed in the C-terminus of Abf1 and Rap1, because this increases the probability that the epitope will be outside of the folded polypeptide without compromising their function (Jarvik and Telmer 1998). However, *C. glabrata* Abf1 and Rap1 have conserved domains at their C-terminus, (Figure S1A & B), which are probably important for the silencing function in *C. glabrata* like in *S. cerevisiae*. In *S. cerevisiae* Abf1 and Rap1 are essential

DNA-binding proteins, which have roles in the activation of transcription of hundreds of genes (Lieb *et al.* 2001), DNA replication and silencing (Shore 1994; Morse 2000; Yarragudi *et al.* 2007; Schlecht *et al.* 2008). It has been shown that these functions depend on C-terminal, conserved domains, present in both Abf1 and Rap1 (Figure S1) (Miyake *et al.* 2002; Ganapathi *et al.* 2011).

We tagged the Rap1 protein at the C-terminal end, and we can detect the fusion proteins by Western-blot (Figure 2), however, it is possible that, the C-terminal tag in Abf1 and Rap1 affects the functions or stability of both proteins, or that the fusion proteins folds such that the tag is buried so that we were not able to detect the tagged Abf1 proteins in the blot. To determine if the proteins were functional, we assayed the growth rate of our tagged strains in three different experiments: 1) we calculated the doubling times of the tagged strains compared to the parental strain in rich liquid medium at 30°C; 2) we determined the growth on rich, solid media at different temperatures of the tagged strains compared to the parental strain; and 3) we looked at growth of the tagged and the C-terminal truncation alleles *abf1-43* and *rap1-21* strains on 5-FOA plates to measure the silencing function of the tagged proteins (Fig. S1).

5.2. The tagged Rap1 proteins are functional for silencing at the E-Rt but cause temperature-sensitivity phenotypes in *C. glabrata*. The RCTd of Rap1 is essential for the silencing of the subtelomeric region of the E-Rt in *C. glabrata*, as shown by the defective allele *rap1-21*, which is unable to mediate the silencing in this region (De las Peñas *et al.* 2003). None of the C– terminal epitopes of Rap1, affect silencing of the *URA3* gene integrated between *EPA2* and *EPA3* (Fig. 4,

insertion 2 and 3) however, the c-Myc tagged version of Rap1 shows a small reduction in silencing of the *URA3* placed between *EPA3* and the telomere while the FLAG tagged version is not affected (Fig. 4, panel B). This is possibly due to two reasons: 1) the c-Myc tag could create changes in the native protein folding, thus affecting the silencing functions of Rap1 and possibly other functions, or 2) the c-Myc tag is interfering with the interaction between the RCTd of Rap1 and the Sir complex (Fig. 3, insertion 3) or with other Rap1 domains, like the BRCT or MYB domains which are necessary for the activation of transcription of ribosomal and glycolytic genes in *S. cerevisiae* (Kurtz and Shore 1991; Lieb 2001; Mizuno *et al.,* 2004) . In *S. cerevisiae*, temperature-sensitive phenotypes at 37 °C, are caused by point mutations in both, MYB and RCT domains (Kurtz and Shore 1991). The double MYB domains are the essential modules that, when deleted, abolish the vegetative growth of the yeast (Graham *et al.* 1999). *C. glabrata* independent Rap1-c-Myc and FLAG transformants grow as well as the parental in liquid media at 30°C, but exhibit sensitivity when are grown in solid media at 47 °C (Fig 3).

To further explore the possibility that c-Myc, and also FLAG are causing conformational changes or interfering with the domains of Rap1, the molecular models of wild-type Rap1 and Rap1 fused to each epitope, were generated using the Phyre structure predictor, and the models were matched using UCSF Chimera (Pettersen *et al.* 2004; Kelley and Sternberg 2009;) (Fig. S2). According to the models in Fig. S2, a combination of conformational changes and interactions between the tags and the protein domains could be happening. c-Myc is predicted to be in a disorder conformation and extends throughout the protein, which in turn provokes Rap1 to adopt a different structure compared with the parental Rap1 (Fig.

S2, compare A and B), in the non-tagged Rap1, the BRCT and the RCT domains are close to each other, while the MYBd are predicted to be separated by an unstructured linker (Fig. S2A), on the other hand the Rap1-c-Myc fusion has the BRCT and RCT domains positioned differently, compared to the wild-type, close to these domains, the structure predicted is different to the non-tagged Rap1. The double MYB adopts the same conformation as in the native Rap1, in fact the MYB domain is in the same orientation with respect to Rap1 without epitope, and when the tagged and the non-tagged versions of Rap1 are superimposed the MYB domain is the unique region that matches in both structures, with a RMSD between 178 atoms of 0.47 Å (Fig. S2D). The FLAG tag on the other hand adopts a compact-like structure, and shares the first aa with the RCTd to form an alpha helix (Fig. S2C), in this case only the N-terminal BRCT domain matches between the non-tagged Rap1 and Rap1-FLAG with a RMSD between 23 atoms 0.98 Å (Fig. S2E).

These models suggest that the folding of *Cg*Rap1 is influenced differently by both epitopes, on one hand c-Myc provokes the BRCT and RCT domains to adopt a different conformation, since these domains don't match with the wild-type structure when both are superimposed, this in turn leads to temperature-sensitivity when the strains grow at 47 °C, as shown in Fig. 3. On the other hand, Rap1-FLAG has dramatic changes in the RCT domain and MYBd, reflected in the growth assays at 47 °C. Despite the temperature-sensitive phenotypes, the tagged Rap1 proteins could probably be used in ChIP assays, since the silencing at subtelomeric loci mediated by them is almost unaffected (Fig. 4).

5.3. The tagged Abf1 proteins are partially functional and are implicated in the silencing of the *EPA* genes at the E-Rt.

It is not known whether *Cg*Abf1 participates in the silencing of the *EPA* genes at the E-Rt (Juárez-Reyes *et al.* 2012) but in the 5-FOA assays, all the tagged and the truncated alleles of Abf1, had an effect in the *URA3* insertions near *EPA1* (between *EPA1* and *EPA2*), here, the level of silencing in the parental *C. glabrata* strain is reduced compared to insertions closer to the telomere, but it completely disappears in independent strains carrying Abf1-c-Myc/FLAG or the *abf1-43* allele (Fig. 5A-B), suggesting that one of the functions of Abf1 in *C. glabrata* is to maintain the repressive chromatin near *EPA1*, although it is not clear how the disruption of Abf1 causes the loss of repression locally.

In *S. cerevisiae* the subtelomeric silencing is established and maintained by the binding of Rap1 to the telomeric repeats, which recruits the Sir proteins (Rusche *et al.* 2003), silencing in *S.cerevisiae* is discontinuous, and only a small group of subtelomeric genes is repressed in a Sir-dependent manner. The silenced genes encoding metabolic enzymes, are located nearby a CREs called core X, where Abf1 and ORC bind and where the Sir proteins enrichment is confined (Ellahi *et al.* 2015). Presumably, these metabolic genes could be expressed under certain conditions, similar to what we have found in *C. glabrata*, where silencing is discontinuous at the E-Rt and some members of the *EPA* family can be expressed under some conditions such as oxidative stress, in spite of a functional subtelomeric silencing of the region (De Las Peñas *et al.* 2003; Domergue *et al.* 2005; Rosas-Hernández *et al.* 2008; Ellahi *et al.* 2015; Juárez-Cepeda *et al.* 2015).

Apparently, there are no core X elements at the E-Rt of *C. glabrata*, although, putative binding sites for Abf1 and ORC, which constitute the core X of *S. cerevisiae* have been detected (Louis 1995) (Fig. 6). These sites are not in the typical array observed at the core X, but are close to each other, and importantly, the effect of complete loss of silencing close to *EPA1* observed in the tagged and defective Abf1 versions, correlates with the putative binding sites of Abf1 found nearby (Fig.6). Perhaps the silencing of the reporter gene in the Abf1-defective strains observed near *EPA1* is the silencing effect mediated by Abf1, which could bind to DNA regions to recruit the Sir proteins. Rap1 has a much stronger effect in silencing at the E-Rt, than Abf1; indeed, in the *rap1-21* mutant almost all the silencing is lost at the three positions tested in this telomere, however, in the *rap1-21* strain, there is a residual silencing between *EPA2* and *EPA3* (insertion 2), and we consider that this residual silencing in the *rap1-21* strain at insertion 2 could be an effect of Abf1 (Fig. 5). To further explore this possibility we generated a double mutant of *C. glabrata* carrying the truncated alleles *rap1-21* and *abf1-43*.

Surprisingly, silencing of the *URA3* in insertion 2 in the double mutant *rap1-21*/*abf1-43*, is the same as in the single *rap1-21* mutant, suggesting that Abf1 at least in this region does not bind or plays a minor role in the maintenance of silenced chromatin, where possibly other proteins also participate. The silencing between *EPA3* and the telomere remained the same in the *rap1-21*/*abf1-43* strain, since the *rap1-21* mutant already shows complete loss of silencing at this position. However, an additive effect between Abf1 and Rap1 is observed in an independent chromosome, at the I-Rt, where the residual silencing observed between *EPA4* and *EPA5* mediated by *rap1-21* is modestly reduced when additionally *C. glabrata*

carries the defective allele *abf1-43* (Fig. 5C-D, insertion 4). Nevertheless the reduction in the silencing at insertion 4 of I-Rt could be an effect of the reduced growth ability of the double mutant, (Fig. 5, *rap1-21*/*abf1-43* see SC plates), which is also exhibited by the double mutant *rap1-21*/*abf1-43* in insertion 2 (Fig. 5B, SC plates). Therefore, it is possible that this efect does not reflect the silencing state at those regions.

5.4. Abf1 and Rap1 could participate in the formation of a telomere loop.

How is Abf1 contributing to the silencing of the *EPA* genes? A possibility is that Abf1, like other silencing proteins, such as the yKu complex and Rif1, is necessary to different extents depending of the particular telomere, thus recruiting other silencing proteins, like the Sir complex, to subtelomeric regions (De Las Peñas *et al.* 2003; Castaño *et al.* 2005; Rosas-Hernández *et al.* 2008). Another possibility is that the effect observed near the E-R and I-R telomeres, probably is a consequence of telomere looping (t-loop), which depends on Sir proteins (de Bruin *et al.* 2001; Zaman *et al.* 2002). A possible model is that Abf1 bound near *EPA1* at the E-Rt, recruits the Sir proteins, which have affinity for Rap1 bound to the telomeric repeats, allowing the formation of a t-loop (Fig.S3). On the other hand, at the I-Rt the silencing depends almost entirely on Rap1 (De Las Peñas *et al.* 2003), but it is possible that Abf1 binds between *EPA4* and *EPA5*, thereby contributing to silencing at this region (Fig. 5 C-D, Fig. 6). This is consistent with the evidence that only in the absence of any of the *SIR* genes (*2-4*), there is a complete loss of silencing at the E-R and I-R telomeres. Thus, Rap1 would be playing a strong role in subtelomeric silencing, mostly, close to the E-R and I-R telomeres and Abf1

would exert its effect in distant regions away from telomere, such as near *EPA1* (Fig. 5 and Fig. S3). This also suggests that, Sir proteins are enriched at discrete regions at the E-Rt of *C. glabrata* in a similar way to what occurs in the core X of the *S. cerevisiae* telomeres (Ellahi *et al.* 2015). The residual silencing between *EPA2* and *EPA3* in the *rap1-21* strain could be due to ORC binding to the putative sites detected (Fig. S3), ORC could probably recruit the Sir proteins since there is evidence that Sir3 interacts with the ORC complex (Gavin *et al.* 2005; Lambert *et al.* 2010; Szklarczyk *et al.* 2015).

5.5. Regulation of the *EPA* genes.

The regulation of the expression of the *EPA* genes is composed of several layers, and one of them is the repression by folded structures, formed at yeast telomeres (de Bruin *et al.* 2000). This mechanism depends on DNA binding proteins that can recruit the Sir proteins, which in turn mediate the interaction at telomeres, and consequently exert the repression at a distance (de Bruin *et al.* 2000; de Bruin *et al.* 2001; Zaman *et al.* 2002). Abf1 is a good candidate in the nucleation of the Sir machinery at E-Rt, since a malfunction on the Abf1 protein causes the loss of silencing near *EPA1*, similar to what happens when *SIR* genes are deleted. This is consistent with Abf1 recruiting Sir at I-Rt as well, where the residual silencing is reduced in the double mutant *abf1-43*/*rap1-21* (Fig. 5 & Fig. S3). It is very important also to note that the elimination of the t-loop regulation abolishes the repression but does not ensure the complete expression of the *EPA* genes. It has been shown that *EPA* genes are induced or repressed differentially, under a variety of conditions and telomere-independent mechanisms, for example:

EPA1 is expressed *in vivo* under limitation of nicotinic acid in a Sir dependent manner; *in vitro*, it is transcribed in lag phase cells but is repressed immediately by a Sir-independent, repressive CRE, the NE (Domergue *et al.* 2005; Gallegos-García *et al.* 2012). *EPA2* is induced *in vitro* by reactive oxygen species as H_20_2, and this expression depends on the redox-responsive transcription factors Yap1 and Skn1, even in the presence of the Sir proteins (Juárez-Cepeda *et al.* 2015). *EPA3* is induced by osmotic stress, glucose starvation and during biofilm formation (Roetzer *et al.* 2008; Kraneveld *et al.* 2011) and basal transcription is observed when the Sil2126 element and the yKu proteins are deleted from *C. glabrata* (Juárez-Reyes *et al.* 2012); the conditions in which *EPA4* and *EPA5* are expressed remain unknown. The regulatory mechanisms of the *EPA* genes are variable, and provide an advantage to the pathogen to persist in the changing environmental conditions of the host. The t-loop formation could be a feasible system dependent on specific DNA sequences and proteins bound to them, to control in part, the expression of a set of genes located near telomeres in some eukayotic pathogens such as *C. glabrata*.

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7. Tables, figure legends, figures and supplementary information

7.1. Tables

Table 1. Doubling times of the C.glabrata BG2 wild-type and the derived strains carrying the tagged Abf1 and Rap1 proteins.

Strain	Doubling times (minutes)[a]
BG2 (parental)	50.3 ± 1.4
Abf1-c-Myc (1)[b]	49.5 ± 1
Abf1-cMyc (2)	47.7 ± 1.1
Abf1-FLAG (1)	46.9 ± 2.2
Abf1-FLAG (2)	48.4 ± 1.3
Rap1-c-Myc (1)	52.1 ± 3.2
Rap1-c-Myc (2)	44.4 ± 1.8
Rap1-FLAG (1)	50 ± 3.7
Rap1-FLAG (2)	49.1 ± 0.8

a. The growth was automatically recorded every 30 min at 30 ° C (see methods).

b. The doubling times of two independent transformants designated as 1 and 2 are shown.

7.2. Figure Legends

Figure 1. Epitope-tagging, complementing vectors and transformation in *C. glabrata*.
(A) Tagging of the *ABF1* and *RAP1* genes. Each construction carries, c-Myc or FLAG epitopes, a resistance marker to Nourseothricin antibiotic (NAT) flanked by 34 bp FRT sites with the PCR products of the C-terminal (COOH) and the 3' Untranslated Region (3' UTR) of each native *C.glabrata* gene (BG2 strain) (see methods). Transformants (A-U) were diagnosed for the integration using primers flanking the specific allele (outside from the cloned fragments). In this scheme only the *ABF1*-FLAG version is shown, but we followed the same strategy for the rest of the constructs. The band size expected for the parental and for the transformants is shown below the gel. **(B)** The complementing vectors contain the 5'UTR, 3'UTR, the ORF of *ABF1* or *RAP1* respectively, a *URA3* selectable marker, an ARS and a *C. glabrata* CEN. These constructs were transformed as supercoiled plasmids. MM, Molecular Weight Marker: 1 kb Plus DNA ladder; CTRs, controls; WT, Wild Type; n, negative control.

Fig. 3. Detection by Western blot of tagged proteins. Immunoblotting of 50 µg of Abf1 and Rap1 in the soluble fraction of *C. glabrata* yeast extracts (see methods). **(A)** PVDF membrane incubated with the αc-Myc. The first two lanes correspond to the positive, Hst1-c-Myc (74 kDa) protein and negative (protein extracts from the parental strain, BG2 untagged) controls, respectively. **(B)** PVDF membrane incubated with the αFLAG. Sum1-FLAG (83 kDa) protein was used as a positive control. Samples; 1-7, were loaded after the controls, and in the order that appear at the right side of the picture. The numbers 1 and 2 within parentheses denote two independent transformants. The expected size of Abf1-c-Myc is 69kDa, Abf1-FLAG is 57 kDa but were not detected (the position is indicated with a red arrow). The expected size of the Rap1-c-Myc fusion is 94 kDa and Rap1-FLAG is 82 kDa (indicated with yellow arrows). CTRs, controls; TP, tagged-protein; UP, untagged-protein.

Fig. 4. Growth of *C. glabrata* strains with tagged and defective Abf1 and Rap1 proteins.
Growth of *C. glabrata* on rich solid media under four different temperatures (see methods). BG2 strain is the parental strain, *rap1-21* is a strain carrying a deletion of the C-terminal 28 amino acids of Rap1, and *hdf1*Δ (*HDF1* encodes yKu70 protein), were used as a negative growth controls.

Fig. 4. Strains carrying Rap1 tagged constructs are able to silence efficiently the *URA3* reporter close to the E-Rt. (A) Schematic representation of the position of two independent insertions of Tn*7* (containing the *URA3* reporter gene) along the E-Rt of *C. glabrata*. **(B)** Defective allele of *rap1-21* was used as a control of loss of silencing, also we tested an analogous mutant, *abf1-43*. c-Myc and FLAG tagged *RAP1* constructs were introduced in each of the strains carrying the *URA3*, insertions 2 and 3 (Panel A).

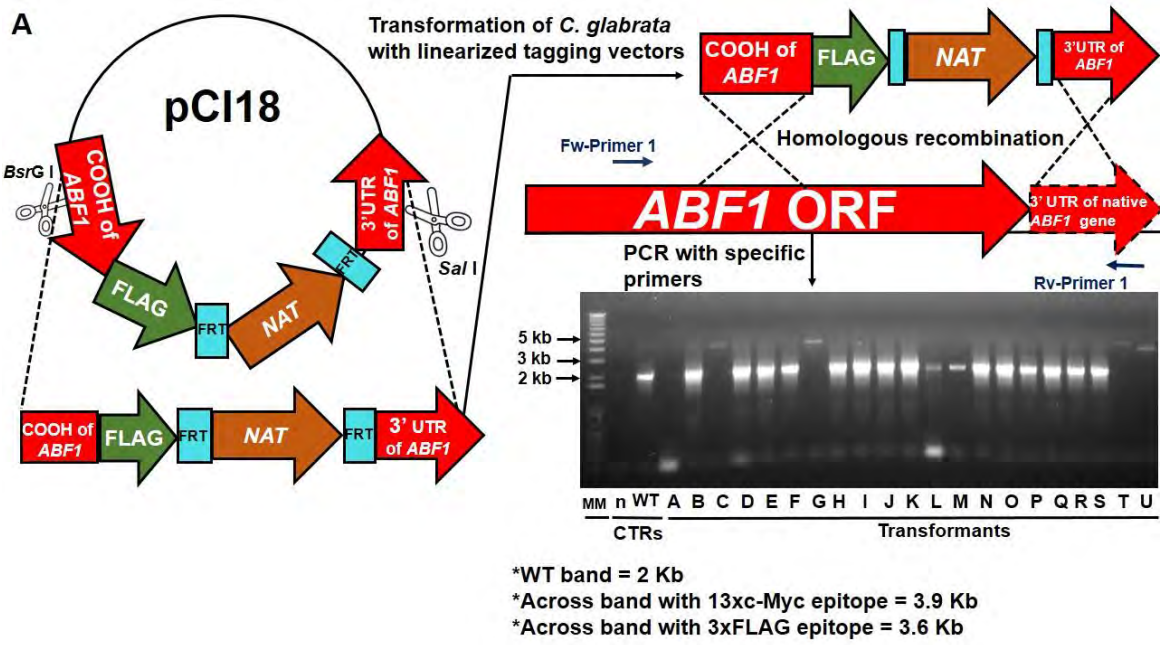
Fig. 5. Abf1 participates in the silencing of the *EPA* genes at the subtelomeric region of the E-R and I-R telomeres. (A) Schematic representation of the position of three independent insertions of Tn7 along the E-Rt of *C. glabrata*. **(B)** In order to investigate if Abf1 is associated with the silencing of the *EPA* genes at E-Rt, the silencing mediated by our tagged Abf1 and the partial allele *abf1-43* as well as the double mutant *rap1-21*/*abf1-43* strain was examined. **(C)** Schematic representation of the *URA3* reporter gene integrated between *EPA4* and *EPA5* in I-Rt. **(D)** The silencing mediated by the C-terminal truncated Abf1 and Rap1 proteins was assayed in the I-Rt.

Fig. 6. **Binding sites for silencing proteins at *C. glabrata* subtelomeric regions detected by MAST and FIMO tools. (A)** Schematic representation of the E-Rt where several putative binding sites for Abf1, ORC and Rap1 proteins were identified using MAST (see materials and methods). **(B)** Putative binding sites for the proteins of interest using FIMO. The Rap1 site within the Sil2126 element reported by Juárez-Reyes *et al.* 2012 wasn´t detected in the MAST scan, only with FIMO was matched. **(C)** At the I-Rt, the Abf1 binding sites were not detected with MAST, so we used FIMO to detect the individual matches within a provided collection of *S. cerevisiae* Abf1 motifs. In this way FIMO detected 7 putative binding sites for Abf1 between *EPA4* and *EPA5*. On the other hand, both MAST and FIMO matched Rap1 binding sites enrichment at the 3' of the ORFs of *EPA4* and *EPA5*. Note that the hypothetical binding sites for the proteins of interest detected by MAST are also detected by FIMO.

The orientation of transcription is represented by the dark grey arrows, the distances from the telomere are indicated and a color code for each protein binding site is at the left of the schemes.

7.3. Figures

Figure 1.



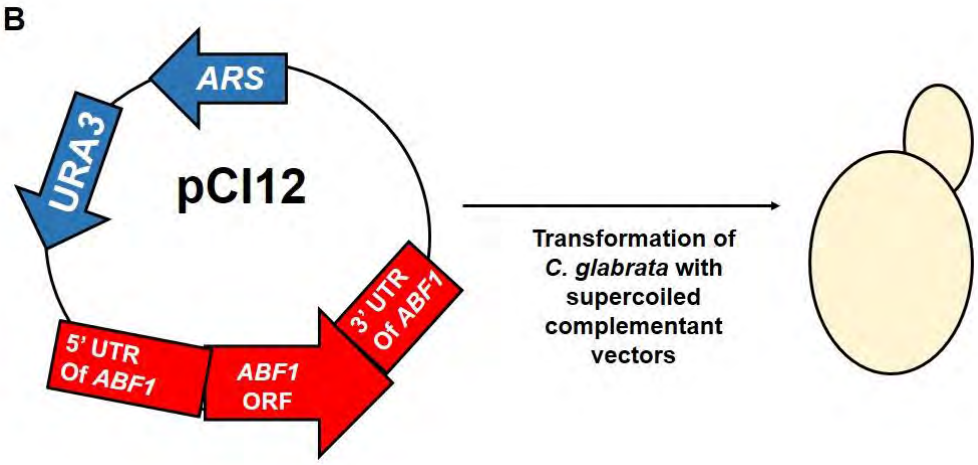
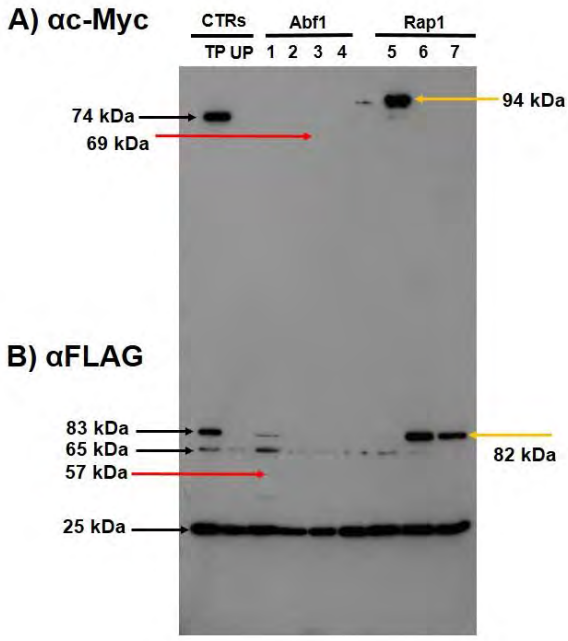


Figure 2.



Lane	Transformant
1	FLAG (1)
2	FLAG (2)
3	cMyc (1)
4	cMyc (2)
5	cMyc (1)
6	FLAG (1)
7	FLAG (2)

Figure 3.

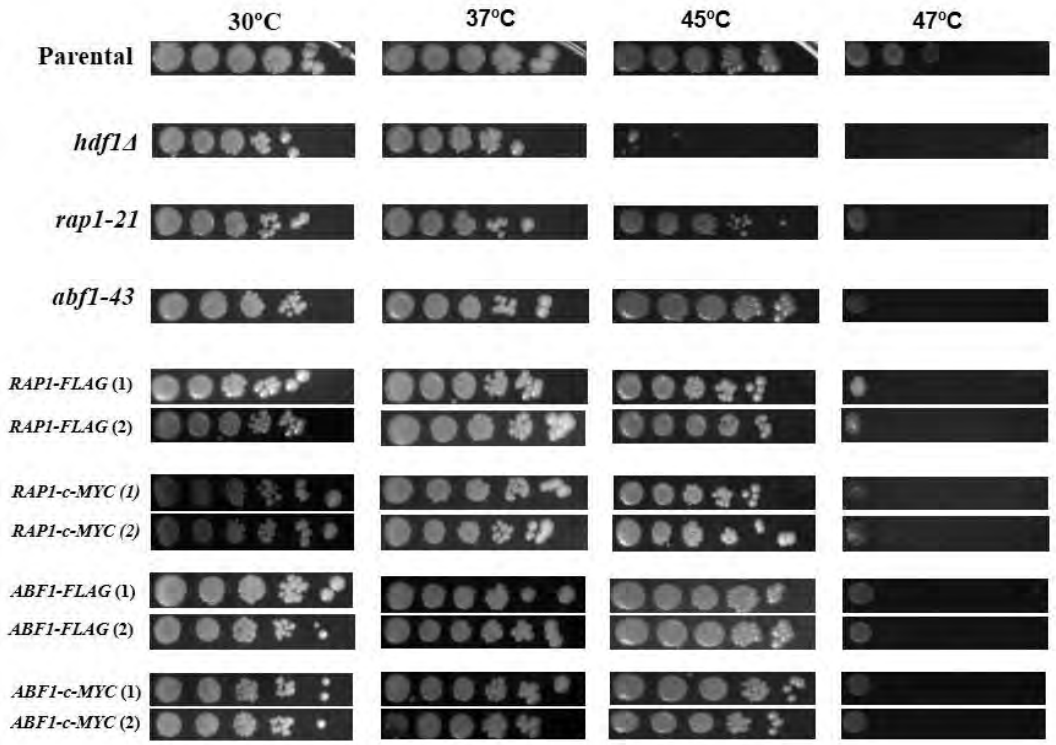

30°C
37°C
45°C
47°C
Parental
hdf1Δ
rap1-21
abf1-43
RAP1-FLAG (1)
RAP1-FLAG (2)
RAP1-c-MYC (1)
RAP1-c-MYC (2)
ABF1-FLAG (1)
ABF1-FLAG (2)
ABF1-c-MYC (1)
ABF1-c-MYC (2)

Figure 4.

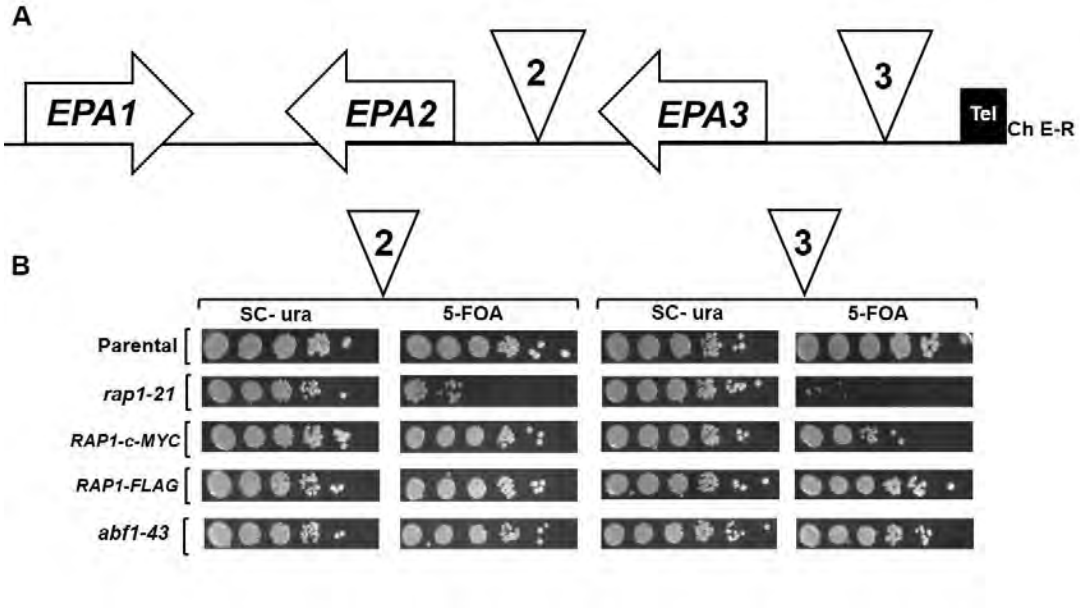

A
EPA1
EPA2
2
EPA3
3
Tel
Ch E-R
B
2
3
SC- ura
5-FOA
SC- ura
5-FOA
Parental
rap1-21
RAP1-c-MYC
RAP1-FLAG
abf1-43

Figure 5.

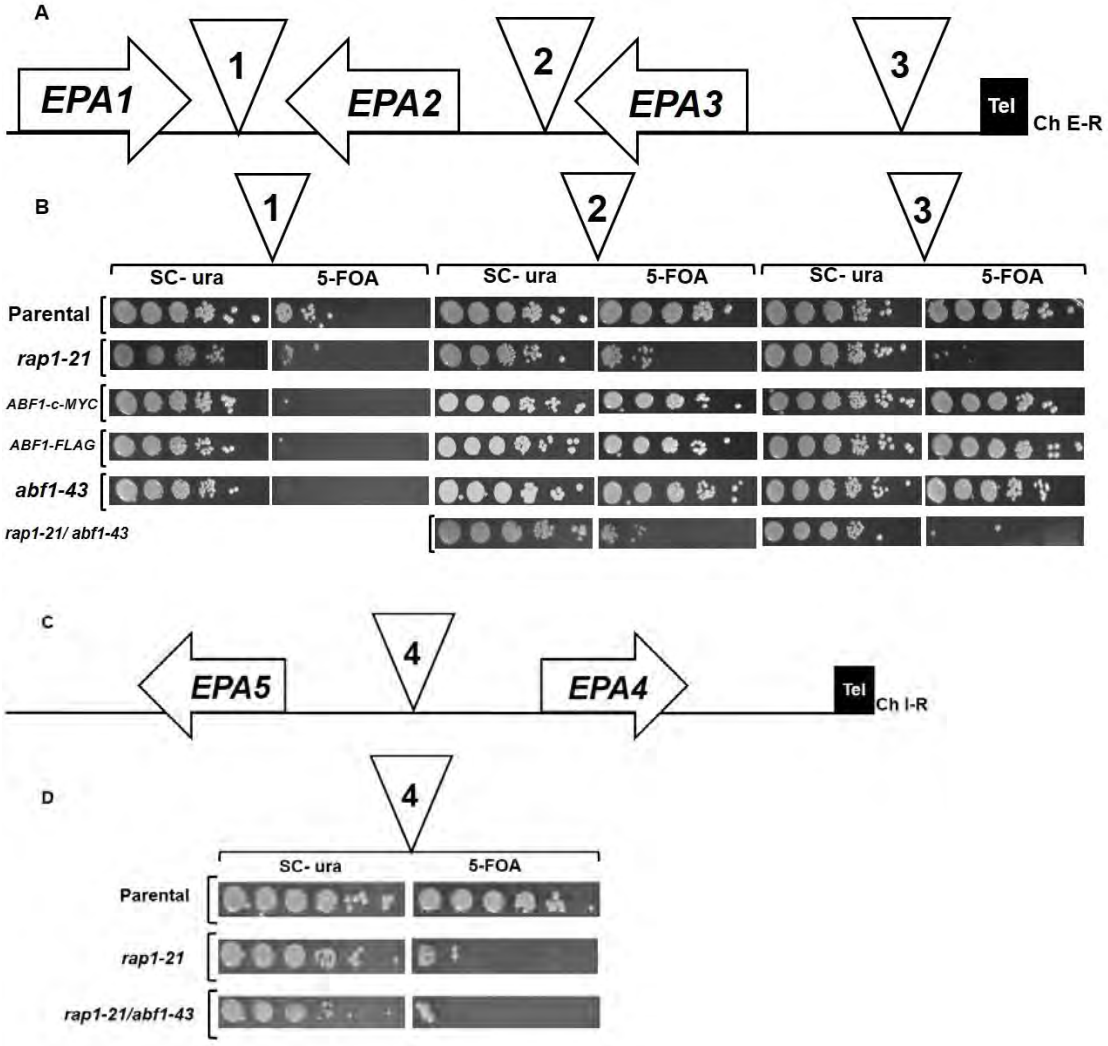
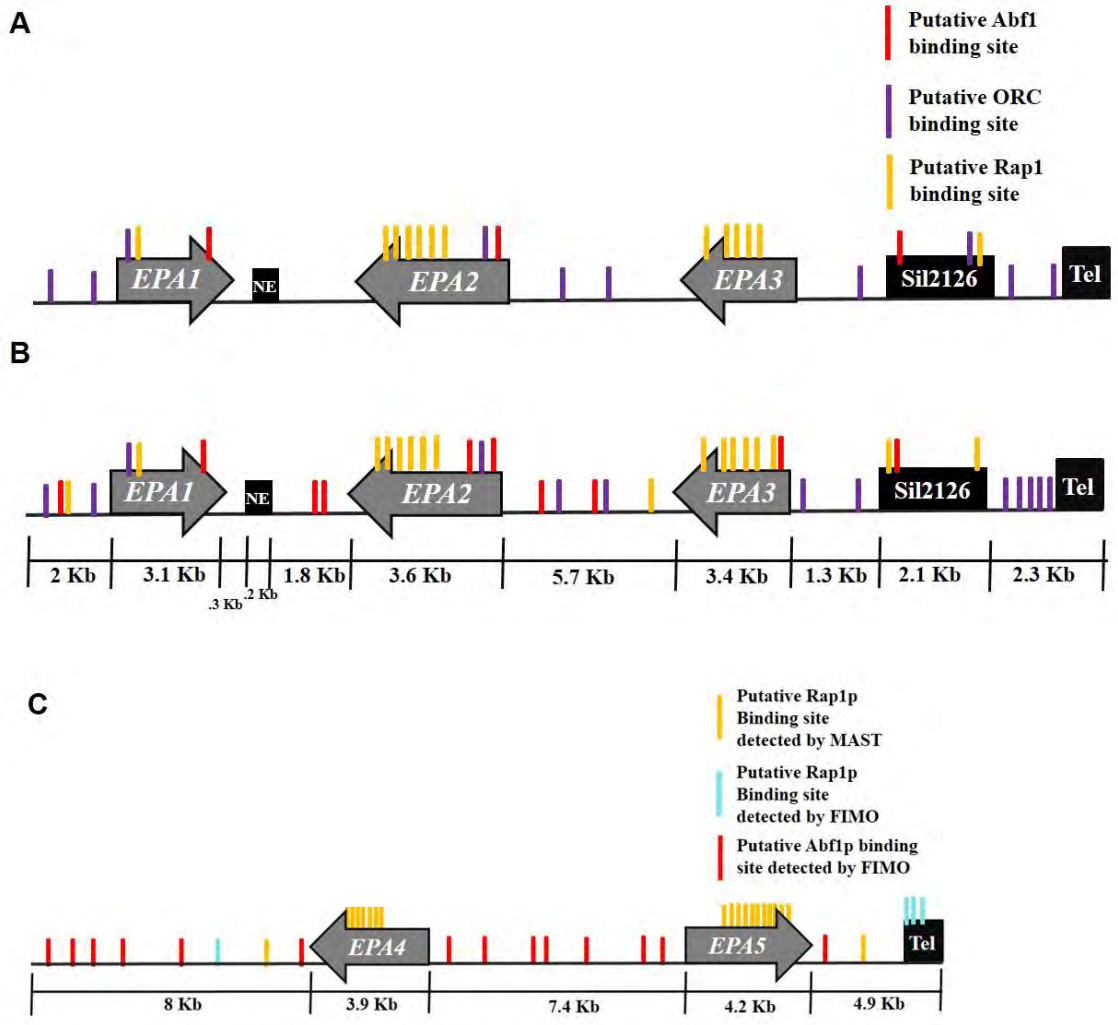

A
EPA1
1
EPA2
2
EPA3
3
Tel
Ch E-R
B
1
2
3
SC- ura
5-FOA
SC- ura
5-FOA
SC- ura
5-FOA
Parental
rap1-21
ABF1-c-MYC
ABF1-FLAG
abf1-43
rap1-21/ abf1-43
C
EPA5
4
EPA4
Tel
Ch I-R
D
4
SC- ura
5-FOA
Parental
rap1-21
rap1-21/abf1-43

Figure 6.


A
Putative Abf1 binding site
Putative ORC binding site
Putative Rap1 binding site
EPA1
NE
EPA2
EPA3
Sil2126
Tel
B
EPA1
NE
EPA2
EPA3
Sil2126
Tel
2 Kb
3.1 Kb
.3 Kb
.2 Kb
1.8 Kb
3.6 Kb
5.7 Kb
3.4 Kb
1.3 Kb
2.1 Kb
2.3 Kb
C
Putative Rap1p Binding site detected by MAST
Putative Rap1p Binding site detected by FIMO
Putative Abf1p binding site detected by FIMO
EPA4
EPA5
Tel
8 Kb
3.9 Kb
7.4 Kb
4.2 Kb
4.9 Kb

7.4. Supplementary Tables

Table S1. *C. glabrata* and *E. coli* strains used in this study.

***E.coli* strain**	**Genotype**		**Use**	**Reference**
DH10B	F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) □80d*lacZ*Δ*M15* Δ*lacX74 deoR recA1 endA1 araD139* Δ(*ara,leu*)7697 *galU galK* □⁻ *rpsL nupG*		Electrocompetent cells	Calvin and Hanawalt 1988
***C.glabrata* strains**	**Parent**	**Genotype**	**Phenotype**	**Reference**
BG2		Clinical isolate		Cormack and Falkow 1999
BG14	BG2	*ura3*Δ::Tn*903* G418^R	Ura$^-$	Cormack and Falkow 1999
***URA3* reporter gene integrated at *EPA* loci**				
CGM147	CGM1	*ura3*Δ::Tn*903* G418^R Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe*I/*Bcg*I). Insertion 1	Ura$^+$	De Las Peñas *et al.* 2003
CGM148	CGM1	*ura3*Δ::Tn*903* G418^R Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$	De Las Peñas *et al.* 2003
CGM149	CGM1	*ura3*Δ::Tn*903* G418^R Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^+$	De Las Peñas *et al.* 2003
CGM160	CGM1	*ura3*Δ::Tn*903* G418^R *rap1-21* Tn*7* at unique region between *EPA5* and *EPA4* (pAP534 *Bcg* I)) Insertion 4	Ura$^+$	De Las Peñas *et al.* 2003
Epitope-tagged proteins integrated in *URA3* reporter strains				
CGM2444	CGM147	*ura3*Δ::Tn*903* G418^R, *ABF1::CMYC*::*nat* (pCI16 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe* I/*Bcg* I). Insertion 1	Ura$^+$ Nat^R	This work
CGM2445	CGM147	*ura3*Δ::Tn*903* G418^R, *ABF1::FLAG*::*nat* (pCI18 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe*I/*Bcg*I). Insertion 1	Ura$^+$ Nat^R	This work
CGM2446	CGM149	*ura3*Δ::Tn*903* G418^R, *ABF1::CMYC*::*nat* (pCI16 / *BsrGI-SalI*) Tn*7* at intergenic region between	Ura$^+$ Nat^R	This work

		EPA3 and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3		
CGM2447	CGM149	*ura3*Δ::Tn*903* G418^R , *ABF1::FLAG*::*nat* (pCI18 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^+$ Nat^R	This work
CGM2581	CGM148	*ura3*Δ::Tn*903* G418^R , *ABF1::FLAG*::*nat* (pCI18 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559/*Eco*RI). Insertion 2	Ura$^+$ Nat^R	This work
CGM2582	CGM148	*ura3*Δ::Tn*903* G418^R , *ABF1::FLAG*::*nat* (pCI18 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559/*Eco*RI). Insertion 2	Ura$^+$ Nat^R	This work
CGM2584	CGM148	*ura3*Δ::Tn*903* G418^R , *ABF1::CMYC*::*nat* (pCI16 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559/*Eco*RI). Insertion 2	Ura$^+$ Nat^R	This work
CGM2585	CGM148	*ura3*Δ::Tn*903* G418^R , *ABF1::CMYC*::*nat* (pCI16 / *BsrGI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559/*Eco*RI). Insertion 2	Ura$^+$ Nat^R	This work
CGM2469	CGM149	*ura3*Δ::Tn*903* G418^R , *RAP1*:: *FLAG*::*nat* (pCI24 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*R I). Insertion 3	Ura$^+$ Nat^R	This work
CGM2470	CGM149	*ura3*Δ::Tn*903* G418^R , *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic región between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^+$ Nat^R	This work
CGM2477	CGM148	*ura3*Δ::Tn*903* G418^R , *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$ Nat^R	This work
CGM2478	CGM148	*ura3*Δ::Tn*903* G418^R , *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$ Nat^R	This work

CGM2480	CGM148	*ura3*Δ::Tn*903* G418^R, *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$ Nat^R	This work
CGM2481	CGM148	*ura3*Δ::Tn*903* G418^R, *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$ Nat^R	This work
Tagged proteins				
CGM2387	CGM1	*ura3*Δ::Tn*903* G418^R, *ABF1::CMYC*::*nat* (pCI16 / *BsrGI-SalI*)	Ura$^-$ Nat^R	This work
CGM2390	CGM1	*ura3*Δ::Tn*903* G418^R, *ABF1::CMYC*::*nat* (pCI16 / *BsrGI-SalI*)	Ura$^-$ Nat^R	This work
CGM2388	CGM1	*ura3*Δ::Tn*903* G418^R, *ABF1::FLAG*::*nat* (pCI18 / *BsrGI-SalI*)	Ura$^-$ Nat^R	This work
CGM2387	CGM1	*ura3*Δ::Tn*903* G418^R, *ABF1::FLAG*::*nat* (pCI18 / *BsrGI-SalI*)	Ura$^-$ Nat^R	This work
CGM2392	CGM1	*ura3*Δ::Tn*903* G418^R, *ABF1* (5'+3' UTR) cloned into pGRB2.0 vector (pCI12)	Ura$^+$	This work
CGM2409	CGM1	*ura3*Δ::Tn*903* G418^R, *RAP1::FLAG*::*nat* (pCI24 / *BsgI-SalI*)	Ura$^-$ Nat^R	This work
CGM2410	CGM1	*ura3*Δ::Tn*903* G418^R, *RAP1::FLAG*::*nat* (pCI24 / *BsgI-SalI*)	Ura$^-$ Nat^R	This work
CGM2411	CGM1	*ura3*Δ::Tn*903* G418^R, *RAP1::CMYC*::*nat* (pCI26 / *BsgI-SalI*)	Ura$^-$ Nat^R	This work
CGM2412	CGM1	*ura3*Δ::Tn*903* G418^R, *RAP1::CMYC*::*nat* (pCI26 / *BsgI-SalI*)	Ura$^-$ Nat^R	This work
CGM2413	CGM1	*ura3*Δ::Tn*903* G418^R, *RAP1* cloned into pGRB2.O vector (pCI28)	Ura$^+$	This work
abf1-43				
CGM2485	CGM147	*ura3*Δ::Tn*903* G418^R (pCI32/PvuII-BsrGI integrated in the chromosome) Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe*I/*Bcg*I). Insertion 1	Ura$^+$ Nat^R	This work
CGM2486	CGM147	*ura3*Δ::Tn*903* G418^R (pCI32/PvuII-BsrGI integrated in the chromosome) Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe*I/*Bcg*I). Insertion 1	Ura$^+$ Nat^R	This work
CGM2488	CGM148	*ura3*Δ::Tn*903* G418^R,	Ura$^+$	This work

		RAP1::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Nat^R	
CGM2489	CGM148	*ura3*Δ::Tn*903* G418^R , *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$ Nat^R	This work
CGM2491	CGM149	*ura3*Δ::Tn*903* G418^R *abf1-43* Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^-$ Nat^R	This work
CGM2492	CGM149	*ura3*Δ::Tn*903* G418^R *abf1-43* Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^+$ Nat^R	This work
rap1-21				
CGM22	CGM1	*ura3*Δ::Tn*903* G418^R rap1-21	Ura$^-$	De Las Peñas *et al.* 2003
CGM151	CGM22	*ura3*Δ::Tn*903* G418^R *rap1-21* Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe* I/*Bcg* I)) Insertion 1	Ura$^+$	De Las Peñas *et al.* 2003
CGM152	CGM22	*ura3*Δ::Tn*903* G418^R *rap1-21* Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*G I/*Sph* I)) Insertion 2	Ura$^+$	De Las Peñas *et al.* 2003
CGM153	CGM22	*ura3*Δ::Tn*903* G418^R *rap1-21* Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst* I/*Eco*R I)) Insertion 3	Ura$^+$	De Las Peñas *et al.* 2003
CGM163	CGM22	*ura3*Δ::Tn*903* G418^R *rap1-21* Tn*7* at unique region between *EPA5* and *EPA4* (pAP534 *Bcg* I)) Insertion 4	Ura$^+$	De Las Peñas *et al.* 2003
rap1-21/abf1-43				
CGM2586	CGM149	*ura3*Δ::Tn*903* G418^R *rap1*-21/abf1-*43* Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^+$ Nat^R	This work
CGM2587	CGM162	*ura3*Δ::Tn*903* G418^R *rap1*-21/abf1-*43* Tn*7* at intergenic region between *EPA3* and telomere (pAP534 *Bam*HI). Insertion 4	Ura$^+$ Nat^R	This work
CGM2588	CGM152	*ura3*Δ::Tn*903* G418^R , *RAP1*::*CMYC*::*nat* (pCI26 / *BsgI-SalI*) Tn*7* at intergenic region between *EPA2* and *EPA3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	Ura$^+$ Nat^R	This work

CG2589	CGM152	*ura3*Δ::Tn*903* G418^R Tn*7* at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	Ura$^+$ Nat^R	This work
***hdf1*Δ**				
CGM78	CGM1	*ura3*Δ::Tn*903* G418^R *hdf1*Δ*::hph* Hyg^R (pAP611 *Bcg* I).	Ura$^-$ Hyg^R	Rosas-Hernández *et al.* 2008

Table S2. Plasmids used in this study.

Plasmid	Relevant genotype	Reference
Cloning vectors		
pGRB2.0	Cloning replicative vector *URA3* Ap^R pRS406::*C.g. CEN* ARS	Zordan et al. 2013
pACYC184	Cloning vector Cm^R Tc^R	Chang *et al. 1978*
pMB11	Cloning vector with an *StuI* restriction site added Cm^R Sac^S	Lab collection
Replicative vectors and epitope-tagging vectors		
pRS306	Integrative vector Amp^R *URA3*	Sikoski *et al.* 1989
pYC10	pCR-TOPO-NAT (flanked by two FRTs) digested with *Sac* I/*Spe* I and filled with T4 polymerase Amp^R	Yáñez-Carrillo *et al.* 2015
pYC14	pYC10 with a *BamH* I restriction site removed and filled with T4 polymerase Amp^R	Yáñez-Carrillo *et al.* 2015
pYC22	pYC14 with a *Sal* I restriction site removed and filled with T4 polymerase Amp^R	Yáñez-Carrillo *et al.* 2015
pYC23	pYC22 digested with *Xho* I and containing the promoter and 3'UTR of the *TEF* gene of *Ashbya gossypii* Vector backbone for the amplification of the NAT gene, [FRT::P_{TEF}::NAT::3'UTR_{TEF}::FRT] Amp^R NAT^R	Yáñez-Carrillo *et al.* 2015
pYC36	A 1.2 kb PCR product amplified from pYC23 (with *BamH* I/*Xho* I cloned on it) corresponding to the *NAT* gene, cloned into the *Stu* I-digested pMB11 vector Cm^R NAT^R	Yáñez-Carrillo *et al.* 2015
pYC40	A 1.2 kb PCR product amplified from pYC23 and digested with *BamH* I/*Xho* I, cloned into the *BamH* I/*Xho* I-digested pRS306 vector Amp^R NAT^R	Yáñez-Carrillo *et al.* 2015
pOZ12	A 0.34 kb fragment (containing *BamH* I/*Bgl* II sites) corresponding to the 3'UTR of the *CTA1* gene of *C. glabrata*, with a FRT sequence cloned into a *BamH* I/*Bgl* II-digested pGEM vector β-lactam^R	Orta-Zavalza *et al.* 2013
pYC44	A 0.34 kb PCR product, amplified from pOZ12, corresponding to the 3'UTR of the *CTA1* gene, flanked by a FRT site, and,	Yáñez-Carrillo *et al.* 2015

	digested with *BamH* I*/Bgl* II*,* cloned into a *BamH* I*/Bgl* II-digested pYC40 modified vector, which has an additional FRT site [FRT::NAT::3'UTR$_{CTA1}$::FRT] Amp^R	
pGE36	¿?	**¿?**
pYC46	A .28 kb fragment digested with *BamH* I*/ Nhe* I released from pGE36, corresponding to a 5 *FLAG* repetitions with the 3'UTR of the *CTA1*, cloned into a *BamH* I*/Nhe* I-digested pYC44 vector Amp^R Nat^R	Yáñez-Carrillo *et al.* 2015
pAP764 vector	¿?	De Las Peñas *et al.* 2003
pOZ14	3'UTR of the *CTA1* gene of *C. glabrata,* released from pOZ12 with *BamH* I*/Bgl* II, cloned into *BamH* I-digested pAP764 vector Hyg^R Amp^R	Orta-Zavalza *et al.* 2013
pAP742 vector	¿?	De Las Peñas *et al.* 2003
pOZ18	A 0.563 kb *BamH* I*/Bgl* II fragment corresponding to 13 *c-Myc* repetitions, released from pAP742, cloned into pOZ12 digested with *BamH* I Hyg^R Amp^R	Orta-Zavalza *et al.* 2013
pYC50	A 0.751 kb *BamH* I/*Nhe I* corresponding to the 13 *c-Myc* repetitions and the 3'UTR of the CTA released from pOZ18, cloned into the pYC44 vector digested with *BamH* I/*Nhe* I	Yáñez-Carrillo *et al.* 2015
pCI1	A 3.07 kb PCR product corresponding to the full lenght *RAP1* gene, cloned into the *Stu* I digested pMB11 vector	This work
pCI3	A 1.05 kb PCR amplified fragment corresponding to the C-terminal of *ABF1*, cloned into the *Stu* I digested pMB11 vector	This work
pCI5	A 1.2 kb PCR product corresponding to the C-terminal of *RAP1*, cloned into the *Stu* I digested pMB11 vector	This work
pCI7	A .45 kb PCR product corresponding to the 3' UTR of *RAP1*, cloned into the *Stu* I digested pMB11 vector	This work
pCI9	A .75 kb PCR product corresponding to the 3' UTR of *ABF1*, cloned into the *Stu* I digested pMB11 vector	This work
pCI10	C-terminal of *ABF1* released from pCI3 with *BamH* I*/Sac* I, cloned into pYC50	This work
pCI12	Full lenght *ABF1* gene released from pCI1 with *BamH* I*/Sac* I, and cloned into the replicative pGRB2.0 vector	This work
pCI13	A 3.07 kb PCR product corresponding to the	This work

	full lenght *RAP1* gene, cloned into the *Stu* I digested pMB11 vector	
pCI14	C-terminal of *ABF1* released from pCI3 with *BamH* I*/Sac* I, cloned into pYC46	This work
pCI16	3' UTR of *ABF1* released from pCI9 with *Kpn* I*/Xho* I, cloned into pCI10	This work
pCI18	3' UTR of *ABF1* released from pCI9 with *Kpn* I*/Xho* I, cloned into pCI14	This work
pCI20	C-terminal of *RAP1* released from pCI5 with *Bgl* II*/Sac* I, cloned into pYC46	This work
pCI22	C-terminal of *RAP1* released from pCI5 with *Bgl* II*/Sac* I, cloned into pYC50	This work
pCI24	3' UTR of *RAP1* released from pCI7 with *Kpn* I*/Xho* I, cloned into pCI20	This work
pCI26	3' UTR of *RAP1* released from pCI7 with *Kpn* I*/Xho* I, cloned into pCI22	This work
pCI28	Full lenght *RAP1* gene released from pCI13 with *EcoR* I*/Kpn* I, and cloned into the replicative pGRB2.0 vector	This work
pCI30	A 0.94 kb PCR product corresponding to the *abf1-43* partial allele and digested with *BamH* I*/Sac* I, cloned into the *BamH* I*/Sac* I digested pYC44 vector	This work
pCI32	3' UTR of *ABF1* released from pCI9 with *Kpn* I*/Xho* I, cloned into pCI30	This work
pCI33	A 1 kb PCR product corresponding to the 5'UTR of *RAP1* and digested with *BamH I/Sac* I*,* cloned into the *BamH* I*/Sac* I digested pYC44 vector	This work
pCI34	A 2.13 kb PCR product corresponding to the 5'UTR of *ABF1* cloned into the *Stu* I digested pMB11 vector	This work
pCI38	5'UTR of *ABF1* digested with *BamHI/SacI* cloned into the *BamH* I*/Sac* I digested pYC44 vector	This work
pCI39	3' UTR of *RAP1* released from pCI7 with *Kpn* I*/Xho* I, cloned into pCI33	This work

Table S3. Oligonucleotides used in this study. The restriction sites added to the primers are indicated in bold

Primer (No.)	Sequence (5'-3')	Site (s) added	Hybridization site (*ABF1*)
1559	GTT**GAGCTC**TTGTGCAGACGATCCGCAGGTCACCG C	*Sac* I	@385 Fw
1560	CTT**GGATCC**CTTGTCCTCTTAATTCAGGTTG	*BamH* I	@1437 Rv
1561	CTT**CTCGAG**GCTCCAATTATTAAAATGAATAAAAGG	*Xho* I	@+13 Fw
1562	CTT**GGTACC**TT**GTGCAG**TGCCGCCAACTTAAGCATA TTATTG	*Kpn* I, *Bsg* I	@+755 Rv
1563	TGTATTCG**GGTACC**GCTAATTCCAG	*Kpn* I	@+933 Rv
1589	CTT**GAGCTC**GATTGTTGTGTAGGCAATATCATAGC	*Sac* I	@-1240 Fw
1590	CTT**GGATCC**CCGAACATTTGGTCAGATCACTG	*BamH* I	@+712 Rv
1834	GGGCCCGCTCCAATTATTAAAATGAATAAAGG	None	@+13 Fw
1835	CTCTGACTCCTCAATCCTTAACC	None	@+1015 Rv
1880	GTT**GGATCC**TTAGACTTCACGAGGAAGCTTGTCGTC GG	*BamH* I	@1308 Rv
1881	CTT**GGATCC**CGTTGTTTGTGTTCTCGTTGG	*BamH* I	@-1 Rv
1884	AGTGCACTTATCCTCCATCC	None	@-2134 Fw
1885	GGATCCACTAGTTCTAGAGCGGCGTTGTTTGTGTTC TCGTTGG	None	@-1 Rv
Primer (No.)	**Sequence (5'-3')**	**Site (s) added**	**Hybridization site (*RAP1*)**
1611	CTT**GGTACC**CGATAAAAATTAGAACCCACTGTG	*Kpn* I	@-578 Fw
1612	CAC**GAATTC**CTCTATGTCACTAAGCTCGCTATC	*EcoR* I	@+455 Rv
1613	CATACAGGTAACTCAATTAGACACAG	None	@812
1614	CTTAGATCTCTATCAGATTTCTCTCCAAAAACTTC	*Bgl* II	@2061
1615	GTTCTCGAGAGAATGAGTGGAGATATTCAGTTTAGA TAAG	*Xho* I	@+1 Fw
1616	CACGGTACCTTCGTGCAGCTCTATGTCACTAAGCTC GCTATC	*Kpn* I, *Bsg* I	@+455 Rv
1617	TCTTTTGCTCGATATCCTCTCC	None	@+687
1618	CCCATGAGACGTACTACACACAC	None	@1751
1836	GTT**GAGCTC**GCTGGAATTTTACCACAGGACTACG	*Sac* I	@-1000 Fw
1837	CTT**GGATCC**GTTCTGGTAAATTAGTGCCACC	*BamH* I	@-66 Rv
1838	GCTGGAATTTTACCACAGGACTACG	None	@-1000 Fw

1839	GGATCCACTAGTTCTCAGAGCGGGATGACTTAAGTT TATAGTCAAGTT ATTTTTTTTTAGG	None	@-1 Rv
1840	GTCCTCGAGGGGGGGCCCGGAGATATTCAGTTTAG ATAAGTTGTTTGG	None	@+9 Fw
1841	CTTCCTCCTCATCTTCTTCTGG	None	@+966
Primer (No.)	**Sequence (5'-3')**	**Site (s) added**	**Hybridization site**
1842	CCGCTCTAGAACTAGTGGATCC	None	Nourseothricin resistance cassette amplification-Fw
1843	GGGCCCCCCCTCGAGGAC	None	Nourseothricin resistance cassette amplification-Rv

7.5. Supplementary Figures

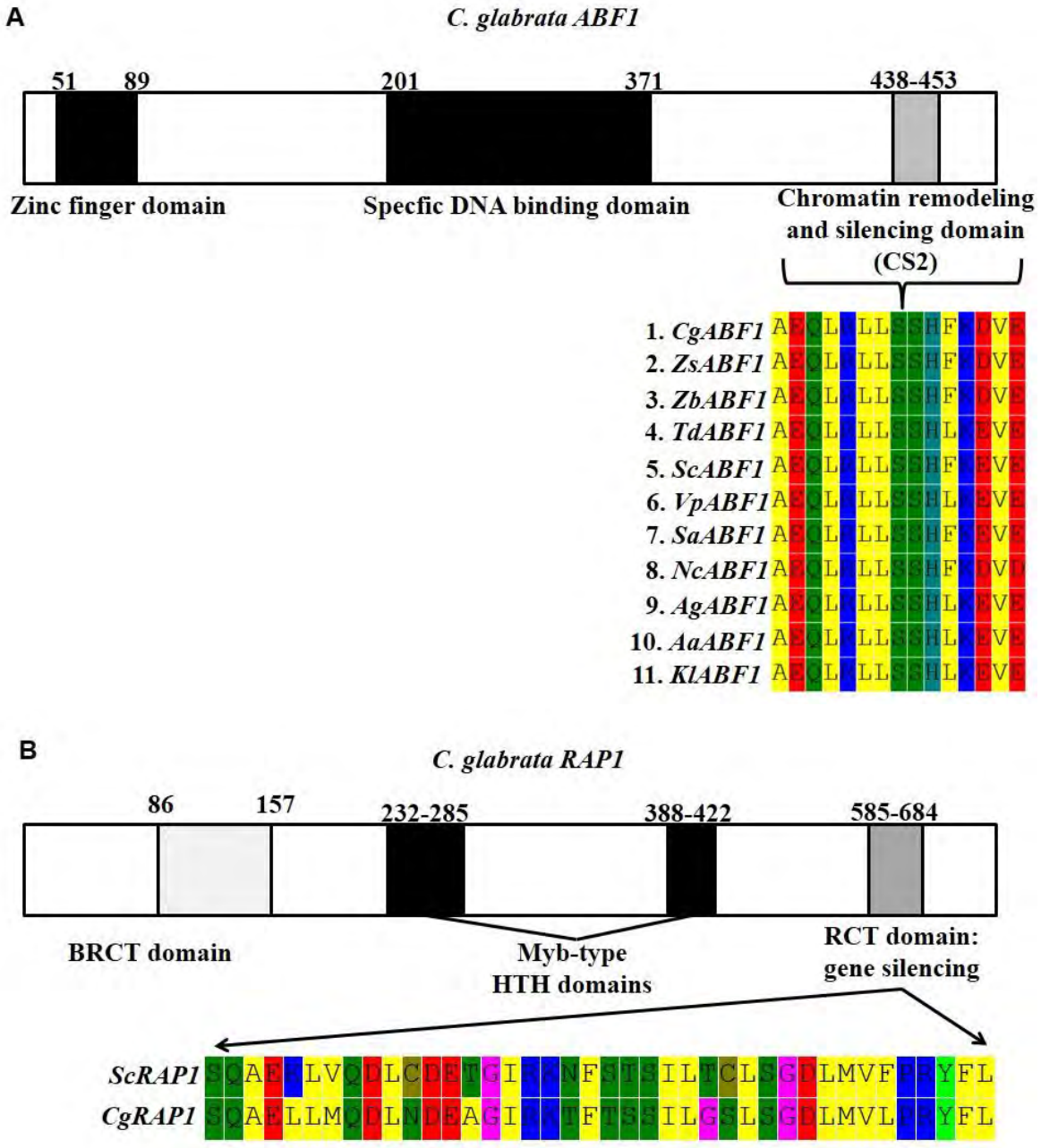


Figure S1. Conserved C' terminal domain in Abf1 and Rap1 proteins. (A) Multiple alignment of Abf1 CS2 domain from different yeast species: **1.** *Candida glabrata* (***Cg***), **2.** *Zygosaccharomyces rouxii* (***Zr***), **3.** *Zygosaccharomyces bailii* (***Zb***), **4.** *Torulaspora delbrueckii* (***Td***), **5.** *Saccharomyces cerevisiae* (***Sc***), **6.** *Vanderwaltozyma polyspora* (***Vp***), **7.** *Saccharomyces arborícola* (***Sa***), **8.**

Naumovozyma castelli (***Nc***), **9.** *Ashbya gossypii* (***Ag***), **10.** *Ashbya aceri* (***Aa***), **11.** *Kluyveromyces lactis* (***Kl***). **(B)** Pairwise alignment of the RCT conserved domain of *C. glabrata* and *S. cerevisiae*. Note that the additional domains involve in DNA binding and other functions are delimited.

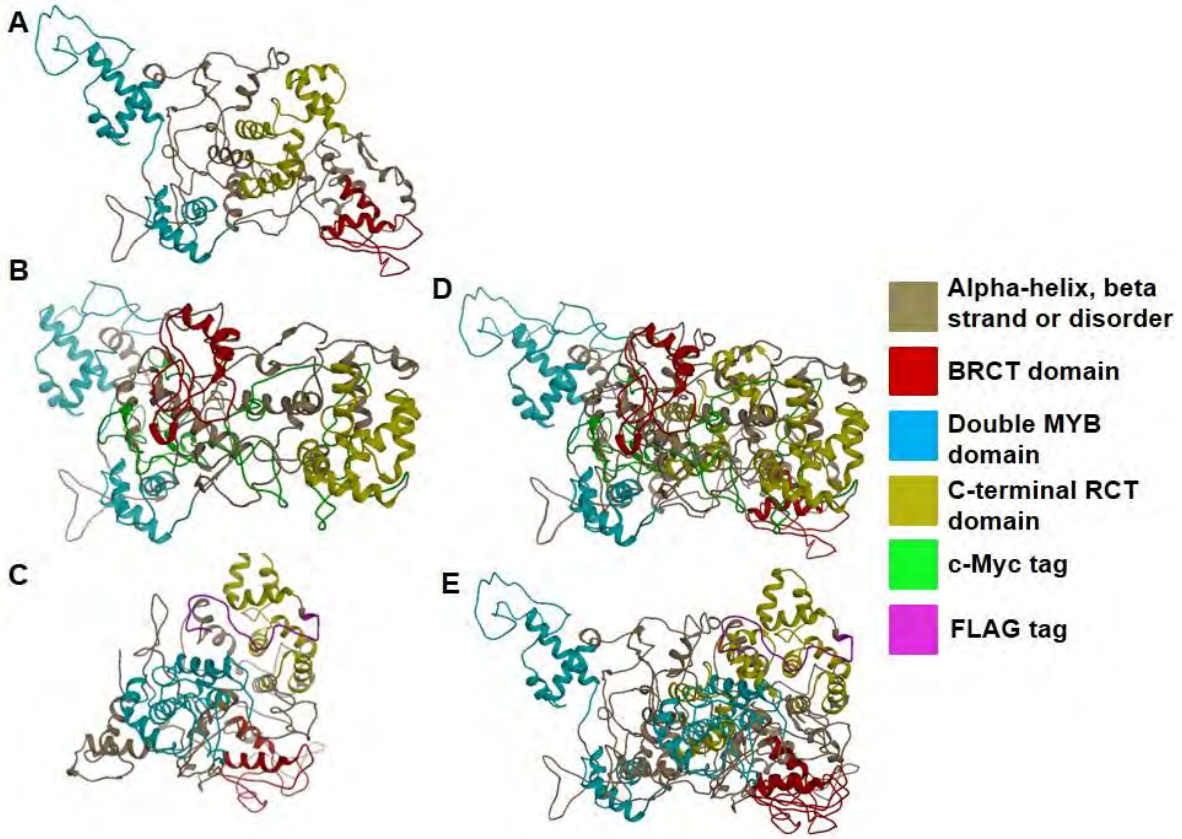


Fig. S2. Protein structures prediction of the *Cg*Rap1 sequence without and with the epitopes. The predicted structures are presented in the same orientation according to the superimposed models generated by UCSF Chimera. **(A)** Structure of Rap1 with a coverage of 70% residues modeled at >90% of confidence, the remaining 30% of the sequence is predicted disordered. **(B)** Predicted structure of Rap1 lacking the stop codon and fused to 13-c-Myc repetitions, colored in green. **(C)** The conformation of the FLAG epitope repeated 3 times (in magenta), is predicted to be part of an alpha helix altogether with the RCT domain. **(D, E)** Superimposition of Rap1 with Rap1-c-Myc and Rap1-FLAG predicted structures.

Each domain of Rap1 is color-coded and the code is at the left of the predicted structures.

Note that there are two DNA binding MYB domains separated by a linker in the parental and in the Rap1-c-Myc.

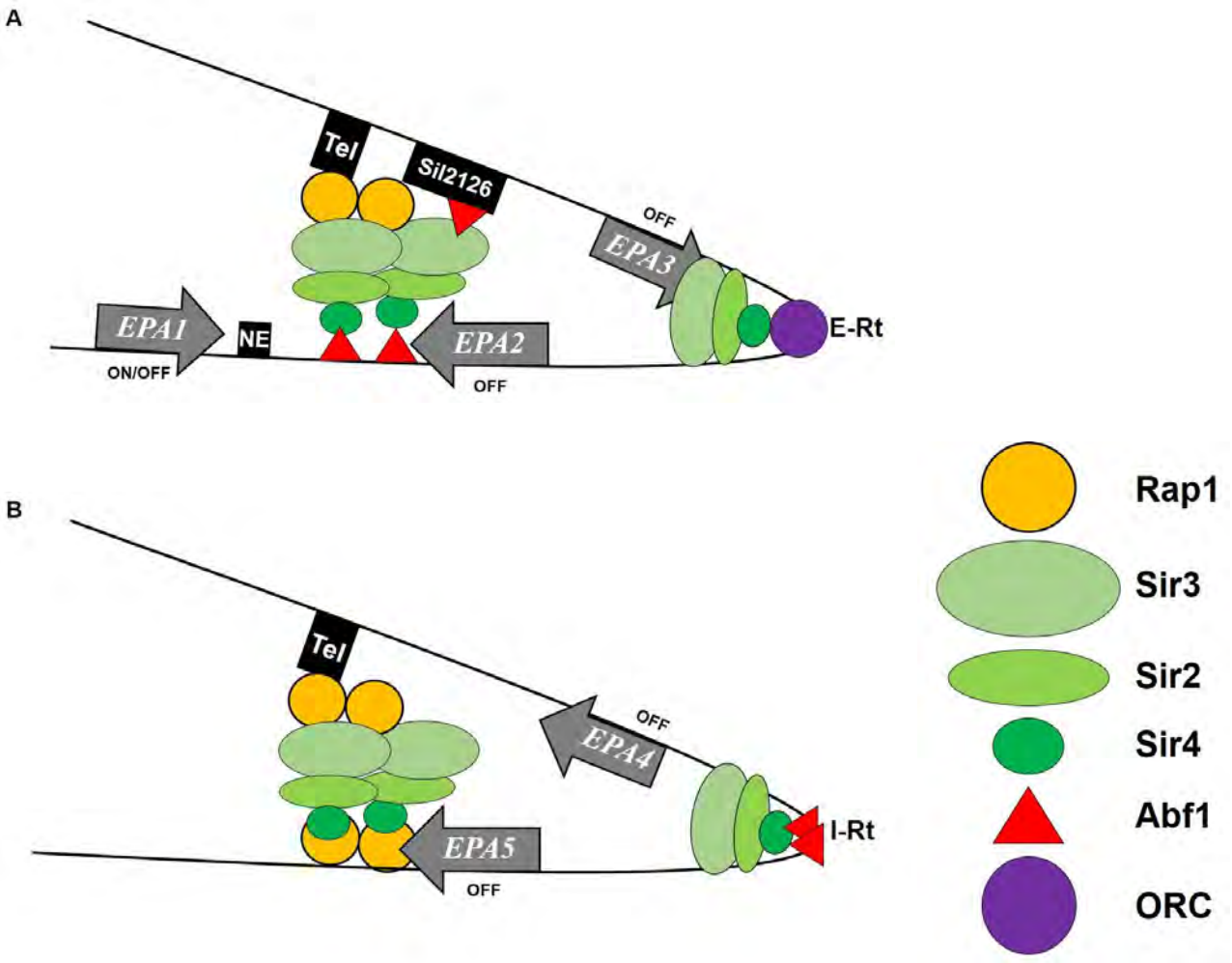


Fig. S3. Model For telomere looping at the E-R and I-R telomeres. (A) Possible t-loop formation at the E-Rt in the wild-type *C.glabrata* BG2 strain. **(B)** In this telomere Abf1 and Rap1 are the interactors proposed to form the T-loop, the disruption of the Abf1 or Rap1 functions (not shown), provokes the T-loop disassembly, still there is residual silencing between *EPA2* and *EPA3*, mediated, possibly, by ORC and the SIR proteins. **(C)** Folding of the I-Rt mediated mainly by Rap1 in the wild type BG2 strain. **(D)** When there is a defect in the silencing functions of Rap1 the t-loop formation is abolished. The residual silencing observed between *EPA4* and *EPA5* could be the effect of Abf1 bound to this region and recruiting the SIR proteins.