ACCELERATED COMMUNICATION

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 292, NO. 15, pp. 6047–6055, April 14, 2017

Functional specialization of two paralogous TAF12 variants by their selective association with SAGA and TFIID transcriptional regulatory complexes

Received for publication, November 30, 2016, and in revised form, February 16, 2017
Published, JBC Papers in Press, March 8, 2017, DOI 10.1074/jbc.C116.768549

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TATA box-binding protein (TBP)-associated factors (TAFs), evolutionarily conserved from yeast to humans, play a central role during transcription initiation. A subset of TAF proteins is shared in transcription factor II D (TFIID) and SAGA transcription regulatory complexes. Although higher eukaryotes contain multiple TAF variants that specify tissue- and developmental stage-specific organization of TFIID or SAGA complexes, in unicellular genomes, however, each TAF is encoded by a single gene. Surprisingly, we found that the genome of *Candida albicans*, the predominant human fungal pathogen, contains two paralogous *TAF12* genes, *CaTAF12L* and *CaTAF12*, encoding H2B-like histone-fold domain-containing variants. Of the available fungal genome sequences, only seven other closely related diploid pathogenic *Candida* genomes encode the two *TAF12* paralogs. Using affinity purifications from *C. albicans* cell extracts, we demonstrate that CaTAF12L uniquely associates with the SAGA complex and CaTAF12 associates with the TFIID complex. We further show that *CaTAF12*, but not *CaTAF12L*, is essential for *C. albicans* growth. Conditional depletion of the two TAF12 variant proteins caused distinct cellular and colony phenotypes. Together our results define a specialized organization of the TAF12 variants and non-redundant roles for the two *TAF12* variants in the unicellular *C. albicans* genome.

Initiation of transcription leading to the formation of a preinitiation complex at the core promoter is a critical control point in gene regulation (1). TFIID[2] is a multisubunit complex that functions as a general transcription factor and contains the TBP and ~14 TBP-associated factors (TAFs) (1, 2). All TAFs are evolutionarily conserved from yeast to humans (3, 4). Barring TAF14, all other TAFs are essential for yeast cell growth; moreover, ~84% of the total yeast genes are dependent on one or more TAFs for their expression in nutrient-rich medium (5, 6). Nine of the TAFs including TAF4 and TAF12 contain a histone-fold domain essential for heterodimerization (reviewed in Refs. 2 and 7). Moreover, TAF5, TAF6, TAF9, TAF10, and TAF12 are also integral components of the yeast SAGA complex (8). The SAGA complex composed of the Spt-Ada-Gcn5-Acetyltranferase subunits is a histone-modifying complex involved in acetylation of histone H3-lysine 9/14 and TBP delivery to facilitate activated transcription, therefore serving a coactivator function (9).

Mechanistically, TAFs have multiple roles in transcription, with their primary role being to deliver TBP to target promoters as part of the TFIID (1). They also function as promoter selectivity factors as part of the TFIID (reviewed in Ref. 10), and as coactivators by mediating interactions between the activators located upstream of the transcription start site and the components of the core transcriptional machinery (11–14). Variants of TBP and several TAFs differentially regulate transcription by virtue of their cell type-, tissue-, or developmental stage-specific expression in higher eukaryotes. In *Drosophila melanogaster*, five TAF paralogs are exclusively expressed in the testis and associate with core TFIID to form testis-specific TFIID (15). In human cells, TAF1L and TAF7L that are specifically expressed during male germ cell differentiation form alternate TFIID complexes to regulate spermatogenesis (16, 17). TAF4b, a paralog of TAF4, is also expressed in a cell type-specific manner (18, 19), and is a part of an alternate TFIID complex that regulates specific sets of genes (20). On the other hand, TAF9 and its paralog TAF9b, although both expressed in multiple cell lines, have limited overlapping functions (21). Indeed, the diverse spectrum of TBP- and TAF-like factors allows the higher eukaryotic cells to form multiple TFIID complexes that differ in their subunit composition, binding specificity, and function (22).

In multicellular organisms, certain TAF variants, such as TAF6L in human SAGA/STAGA (23–25) and the SAF6, which heterodimerizes with TAF9 in *Drosophila* (26), also specifically associate with SAGA complex to carry out tissue-specific functions. TBP or TAF isoforms are not known in unicellular organisms, with the exception of two *TAF5*-like genes SpTAF5 (originally called taf72^{+}) and SpTAF5b (originally called taf73^{+}) in *Schizosaccharomyces pombe* (27, 28). Although both SpTAF5 and SpTAF5b associate with the TFIID, only the SpTAF5b protein is associated with Gcn5 in *S. pombe* (27, 28).

In this study, we report that two novel paralogous *TAF12* genes, *CaTAF12L/orf19.470* and *CaTAF12/orf19.6820*, have non-redundant functions in *Candida albicans*, the most predominant fungal pathogen causing invasive candidiasis in humans (29). Using coimmunoprecipitation assays, we show

This work was partially supported by a grant from the Department of Biotechnology and through the DST-PURSE and UPE2 grants, and departmental funding under DST-FIST and UGC-NRC grants. This work was also supported by junior and senior research fellowships from the Council of Scientific and Industrial Research, New Delhi (to I. S. and P. P.) and by a senior research fellowship from the Indian Council for Medical Research, New Delhi (to S. S.). The authors declare that they have no conflicts of interest with the contents of this article.
This article contains supplemental text, Fig. S1, and Tables S1–S5.

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[2] The abbreviations used are: TFIID, transcription factor II D; TAF, TBP-associated factor; TBP, TATA box-binding protein; HFD, histone-fold domain; TAP, tandem affinity purification; IP, immunoprecipitation; YPD, yeast extract peptone dextrose; YPMal, yeast extract peptone maltose; CFW, calcofluor white; 5-FOA, 5-fluoroorotic acid; Ca, *Candida albicans*.

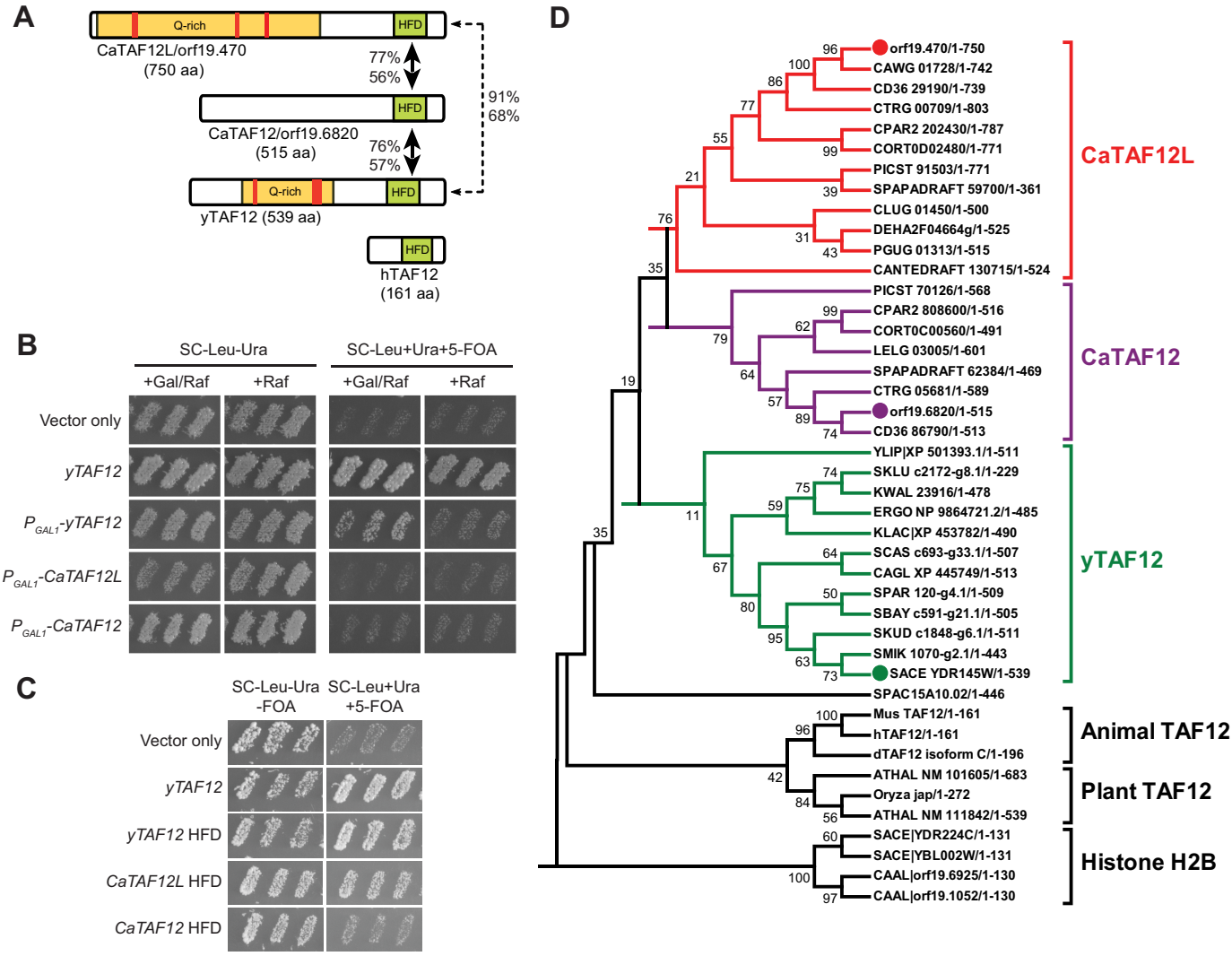


Figure 1. Diploid *Candida* clade genomes encode two TAF12 proteins bearing H2B-like histone-fold domain. *A*, the schematic diagram showing the two *C. albicans* TAF12-like sequences (orf19.470 and orf19.6820), the *S. cerevisiae* TAF12 (yTAF12), and the human TAF12. The *yellow-shaded region* contains abundant Gln residues, and the *red-filled boxes* indicate tandem Gln repeats. The extent of homology (percentage of similarity and identity) of each HFD sequence with respect to the yTAF12 HFD is shown. *aa*, amino acids. *B* and *C*, genetic complementation analysis of *S. cerevisiae taf12*Δ strain by *CaTAF12L* and *CaTAF12*. Shown is the growth phenotype of *S. cerevisiae* strains bearing y*TAF12* (ISC21), *CaTAF12L* (ISC22), or *CaTAF12* (ISC23) under the control of P_{GAL1} promoter in Gal/Raf (activating conditions) and Raf (basal expression) conditions. Yeast strains bearing plasmid-borne *yTAF12* (ISC25) and vector-only (ISC20) were used as controls. *C*, growth phenotype of *S. cerevisiae* strains bearing *TAF12L* or *TAF12* HFD expressed from y*TAF12* promoter. *S. cerevisiae* strains bearing y*TAF12* (ISC25), y*TAF12* HFD (ISC26), *CaTAF12L* HFD (ISC27), or *CaTAF12* HFD (ISC28) or vector alone (ISC24) were tested by replica printing for growth upon eviction of a plasmid bearing y*TAF12* on SC−Leu−Ura and SC−Leu+Ura+5-FOA plates, respectively, at 25 °C. *D*, phylogenetic tree of the TAF12 sequences. A multiple sequence alignment (total taxa = 43) was constructed using TAF12 amino acid sequences from Saccharomycotina species and from higher eukaryotes, as well as the histone-fold domain of histone H2B from *S. cerevisiae* and *C. albicans*. A phylogenetic tree was constructed using the Neighbor-Joining method in the MEGA6 software (56). All default parameters were chosen except that bootstrap test (1000 replicates) was used for the test of phylogeny. The percentage of replicate trees in which the associated taxa clustered together is shown next to the branches. All positions containing alignment gaps and missing data in pairwise sequence comparisons were eliminated. The clusters CaTAF12L, CaTAF12, yTAF12, and animal and plant TAF12 are indicated on the *right*. The histone H2B sequences formed an outgroup. The abbreviated species names in the tree are expanded in supplemental Table S1.

that CaTAF12L is specifically associated with SAGA, and CaTAF12 is specifically associated with the TFIID complex. Although *CaTAF12L* deletion was not lethal, the deletion of *CaTAF12* was lethal in *C. albicans*. Depletion of CaTAF12L and CaTAF12 protein levels caused distinct cellular and colony morphologies. We further show that *CaTAF12L* is required for oxidative stress resistance of *C. albicans*.

Results

C. albicans genome encodes two TAF12 variants

C. albicans genome surprisingly has two ORFs orf19.470 (named here as Ca*TAF12L*) and orf19.6820 (Ca*TAF12*) encoding histone H2B-like histone-fold domain (HF domain) (Fig. 1*A*). The rest of the 13 *TAF* genes are encoded as single ORFs. Each of the two *C. albicans* TAF12 protein sequences have high sequence similarity (identity >56%) with the yTAF12 only in their HF domains. Although both CaTAF12L and CaTAF12 have extended amino-terminal regions, only CaTAF12L contains the Gln-rich region found in *y*TAF12 (Fig. 1*A*). To test whether the two *C. albicans TAF12* genes could function in the budding yeast *Saccharomyces cerevisiae*, we expressed the *CaTAF12L* and *CaTAF12* genes from the yeast *GAL1* promoter. The phenotype data showed that overexpression of neither *TAF12L* nor *TAF12* whole ORFs could complement the

*taf12*Δ deletion (Fig. 1*B*). However, overexpression of *TAF12L* was dominant negative over y*TAF12* and resulted in slow growth (SC−Leu−Ura+Gal/Raf) (Fig. 1*B*). Previous studies showed that the TAF12 HF domain alone was sufficient for *S. cerevisiae* viability (30) and coactivator function in *S. cerevisiae* (31). Therefore we cloned and expressed *CaTAF12L* and *CaTAF12* fragments encoding the HF domain alone from high copy plasmids in the *S. cerevisiae taf12*Δ strain. Although the y*TAF12* histone-fold domain (HFD) fully complemented *taf12*Δ deletion, only *CaTAF12L* HFD, but not that of *CaTAF12*, provided at least partial complementation (+5-FOA; Fig. 1*C*). Thus the HF domains of CaTAF12L and CaTAF12 also seem to have functionally diverged between each other.

BLAST search of the publicly available fungal genome sequences identified two *TAF12*-like ORFs from eight asexually reproducing *Candida* clade species. All other fungal genomes examined, including the sexually reproducing *Candida* clade species, contained a single *TAF12* gene. Among the higher eukaryotic genomes searched, only the *Arabidopsis thaliana* genome contains two *TAF12* genes (32), but their distinct functional roles are not understood. (Fig. 1*D*) To understand the evolutionary history of the two CaTAF12 sequences, the TAF12 amino acid sequences from several genomes were aligned and a phylogenetic tree was constructed. The different TAF12 proteins formed five major clusters with the two TAF12 sequences from *C. albicans* clustering into separate branches occupied by the orf19.470-like (*CaTAF12L*) and orf19.6820-like (*CaTAF12*) sequences (Fig. 1*D*). These analyses showed that although CaTAF12L-like sequences bearing a glutamine-rich amino-terminal region were found in all fungal species examined, the CaTAF12-like sequences were found only in the eight *Candida* clade species (Fig. 1*D*) that are diploid fungal pathogens (33, 34). Moreover, interrogation of the Candida Gene Order Database showed that both *CaTAF12L* and *CaTAF12* genes seem to have lost synteny in all *Candida* clade genomes with reference to the *S. cerevisiae TAF12* gene (35, 36). Therefore we suggest that the two *TAF12* ORFs may have originated within the fungal kingdom during the course of the evolution but perhaps were retained only in the diploid *Candida* clade species in the absence of meiosis.

CaTAF12L associates with SAGA, whereas CaTAF12 associates with TFIID

The architecture of TFIID and the SAGA is governed by multiple TAF-TAF HFD interactions. Although TAF4 and TAF12 form a histone H2A-and H2B-like dimer in TFIID (37), TAF4 is absent in the SAGA complex. In its wake, the TAF12 protein heterodimerizes with Ada1, an H2A-like HFD-containing protein in the SAGA (38). Thus there is a distinct specificity in the interaction of TAF12 with its partner in TFIID and SAGA complexes. Moreover, the observations that TFIID and SAGA complexes each contain two molecules of TAF12 (39–41) suggest that both CaTAF12 homologs may associate with either TFIID or SAGA in two copies. Therefore we tested whether one or both of the two TAF12 variants could associate with the TFIID and/or the SAGA complex in *C. albicans*.

Western blotting analysis of *C. albicans* cell extracts showed that both CaTAF12L and CaTAF12 are expressed constitutively in rich medium (YPD) in wild-type cells (Figs. 2, *A* and *B*, and 3*B*). Therefore homozygous strains bearing either TAP-tagged TAF11, a component of the TFIID complex, or TAP-tagged Ada2, a component of the SAGA complex, in the background of either 3×HA-tagged *CaTAF12L* or 3×HA-tagged *CaTAF12* expressed from their native loci were constructed. Total cell extracts from these epitope-tagged strains were used for coimmunoprecipitation assays. Both Ada2-TAP and TAF11-TAP were efficiently pulled down from the respective cell extracts (Fig. 2, *A* and *B*). Interestingly, CaTAF12L-HA_3 was detected only in the Ada2-TAP pulldown, but not in the TAF11-TAP pulldown (Fig. 2*A*). In contrast, CaTAF12-HA_3 was detected only in the TAF11-TAP pulldown but not in the Ada2-TAP pulldown (Fig. 2*B*). The blots were also probed with polyclonal antibodies against Ada1 and TAF4, the TAF12 dimerization partners in SAGA and TFIID, respectively. We found that Ada1, but not TAF4, was coimmunoprecipitated with Ada2-TAP (Fig. 2*A*), and that TAF4, but not Ada1, was coimmunoprecipitated with TAF11-TAP (Fig. 2*B*).

To independently establish the association of CaTAF12L and CaTAF12 with SAGA and TFIID, respectively, we carried out immunoprecipitation using cell extracts from strains expressing 3×FLAG-tagged Ada1 or TAF4 and probed the Western blots with polyclonal CaTAF12L and CaTAF12 antibodies. Consistent with the TAP immunoprecipitation assays, the CaTAF12L protein exclusively coimmunoprecipitated with Ada1-$FLAG_3$ protein (Fig. 2*C*), whereas the CaTAF12 protein exclusively coimmunoprecipitated with TAF4-$FLAG_3$ protein (Fig. 2*D*). These immunoprecipitation assays established that CaTAF12L and CaTAF12 associate differentially with the two transcriptional regulatory complexes SAGA and TFIID in *C. albicans*.

Next we tested whether one or both of the TAF12-like proteins could interact with TBP. Coimmunoprecipitation assays were conducted using cell extracts from strains expressing either CaTAF12L-HA_3 or CaTAF12-HA_3 in the background of TAP-tagged TBP. Indeed, TBP was coimmunoprecipitated with CaTAF12-HA_3 (ISC34; Fig. 2*E*). However, in the CaTAF12L-HA_3 immunoprecipitation, the amount of TBP recovered was at or close to the background level obtained in the untagged control SN87 cell extract (ISC33; Fig. 2*E*). Previous studies in yeast showed that although TBP can genetically and physically interact with SAGA subunits (42), TBP is not purified as an integral component of SAGA complex (8, 43, 44). Together these biochemical data established an *in vivo* association of CaTAF12L with SAGA complex and CaTAF12with TFIID complex in *C. albicans*.

CaTAF12 but not CaTAF12L is essential for viability of C. albicans

To investigate the *in vivo* functions of *CaTAF12L* and *CaTAF12* genes in *C. albicans*, we constructed *C. albicans* strains bearing glucose-repressible *CaTAF12L* and *CaTAF12* genes, *viz.* P_{MAL2}-*CaTAF12L* and P_{MAL2}-*CaTAF12*, and examined cell growth upon promoter shut-off in YPD medium. Although we did not see any measurable deleterious effect of

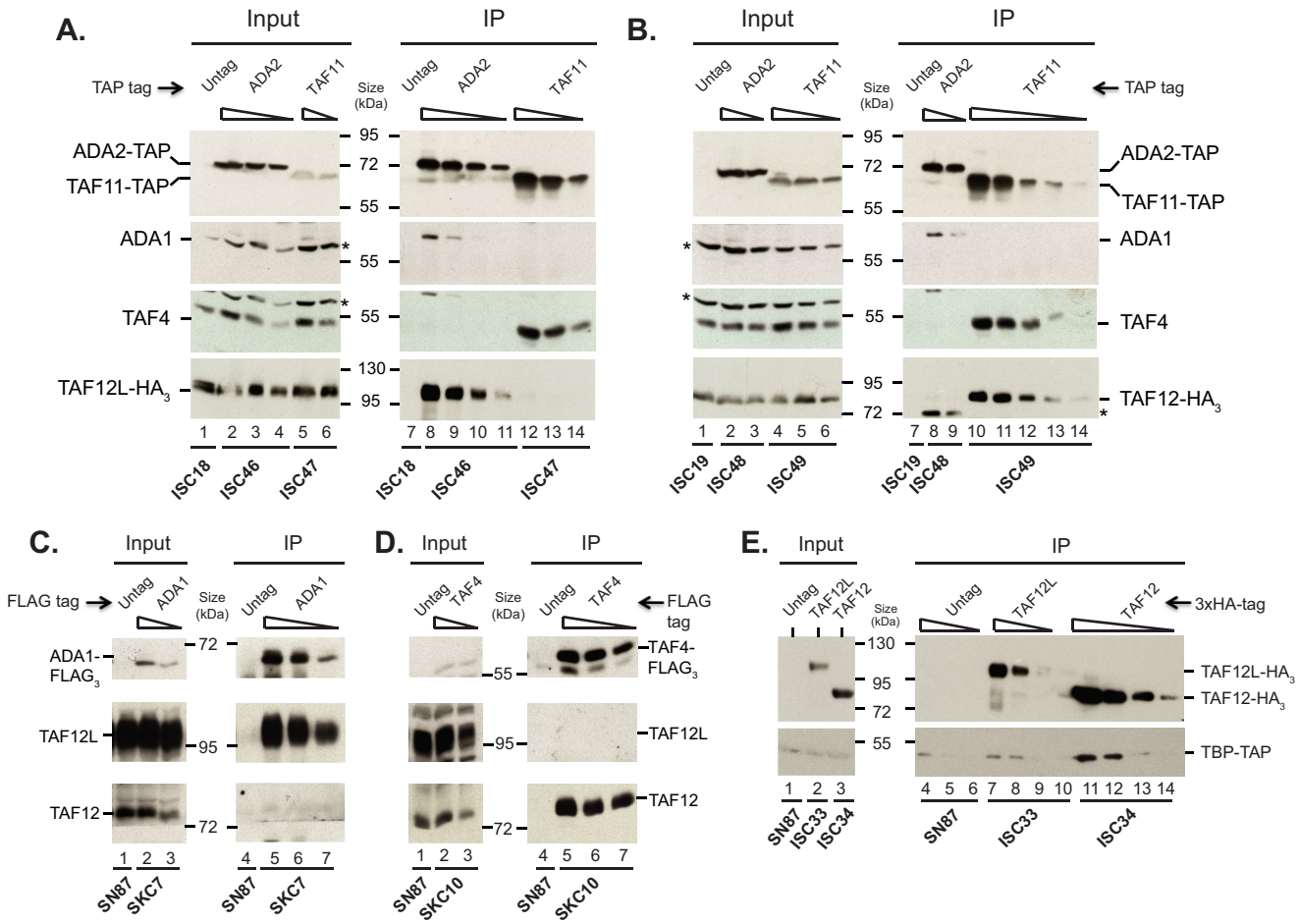


Figure 2. Coimmunoprecipitation of CaTAF12L with SAGA and CaTAF12 with TFIID. *A*, CaTAF12L and Ada1 coimmunoprecipitate with Ada2-TAP but not with TAF11-TAP. *B*, CaTAF12 and TAF4 coimmunoprecipitate with TAF11-TAP but not with Ada2-TAP. Whole-cell protein extracts from control (ISC18 or ISC19) or the various TAP-tagged strains (ISC46-ISC49) were used for immunoprecipitation with IgG-Sepharose beads. 2-fold serial dilutions of the input cell extracts (starting at 100 μg) or the IP eluates (starting at 50% eluate) were blotted, excised into appropriate strips, and probed directly or stripped and reprobed as follows. The TAP-tagged proteins were detected with rabbit IgG Fc fragment, the 3×HA-tagged TAF12L and TAF12 proteins were detected with anti-HA (12CA5, Roche Applied Science) antibody, and the ADA1 and TAF4 proteins were detected with respective mouse polyclonal antibodies in the immunoblot. A nonspecific band detected by the anti-ADA1 antibody is indicated by an *asterisk*; this band was also found in the TAF4 input panels due to incomplete stripping of the blot, which was first probed with anti-Ada1 antibody prior to the anti-TAF4 antibody probe. Similarly, the residual Ada2-TAP band was seen in the IP lanes due to incomplete stripping prior to anti-ADA1, anti-TAF4, and anti-HA probes (*asterisk*; *lanes 8* and *9*). *C* and *D*, coimmunoprecipitation of CaTAF12L with Ada1-FLAG$_3$ (*C*) and CaTAF12 with TAF4-FLAG$_3$ (*D*). Whole-cell protein extracts from control (SN87) or the 3×FLAG-tagged strains SKC7 and SKC10 were used for immunoprecipitation with FLAG-M2 affinity agarose beads (Sigma-Aldrich), and eluates and input whole-cell extracts were analyzed by Western blotting. Anti-FLAG antibody was used to detect Ada1-FLAG$_3$, and mouse polyclonal anti-TAF4 antibody was used to detect TAF4-FLAG$_3$. TAF12L and TAF12 were detected in the immunoblots with respective rabbit polyclonal antibodies. *E*, TBP coimmunoprecipitates with CaTAF12. Whole-cell protein extracts from untagged (SN87) or TBP-TAP strains containing either CaTAF12L-HA$_3$ (ISC33) or CaTAF12-HA$_3$ (ISC34) were used for immunoprecipitation with anti-HA antibody, and the input samples and the IP eluates were blotted as above and probed with rabbit IgG Fc fragment or anti-HA (12CA5) antibodies.

overexpression of *CaTAF12L* from the P_{MAL2} promoter, overexpression of *CaTAF12* caused slight growth defect at 25 °C (YPMal, Fig. 3*A*). Upon promoter shut-off (YPD), the P_{MAL2}-*CaTAF12* strain showed extreme slow growth as compared with the promoter-on condition (YPMal; Fig. 3*A*). In contrast, the P_{MAL2}-*CaTAF12L* strain showed only a slight growth defect upon promoter shut-off (Fig. 3*A*). Western blotting analysis confirmed the depletion of the CaTAF12L and CaTAF12 proteins, respectively, in the P_{MAL2}-*CaTAF12L* and P_{MAL2}-*CaTAF12* strains within 5–6 h of promoter shut-off (Fig. 3*B*). Moreover, depletion of either of the TAF12 variants did not significantly alter the steady-state level of the other TAF12 variant.

To complement the mutant phenotypes of P_{MAL2}-*CaTAF12L* and P_{MAL2}-*CaTAF12* strains in glucose medium, *CaTAF12L* or *CaTAF12*, respectively, was reintroduced from the P_{TET} constructs. Indeed, the P_{TET}-*CaTAF12L* and P_{TET}-*CaTAF12* constructs yielded robust complementation of the defective growth phenotypes of *Cataf12l* (P_{MAL2}-*CaTAF12L*) and *Cataf12* (P_{MAL2}-*CaTAF12*) mutants specifically in doxycycline-containing medium (Fig. 3*C*). The control vector-only transformants did not alter the growth defect (Fig. 3*C*). We also attempted to construct homozygous null mutant strains using different deletion strategies but were unable to obtain *taf12*Δ/Δ mutant strain (data not shown), indicating that *CaTAF12* is essential for *C. albicans* growth. Although the *taf12l*Δ/Δ mutant was viable, the strain exhibited severe slow-growth phenotype (Fig. 3*D*). Expression of *CaTAF12L* from the *TET* promoter complemented the growth defect of the *Cataf12l*Δ/Δ mutant at both 30 °C and 37 °C (Fig. 3*D*). Exam-

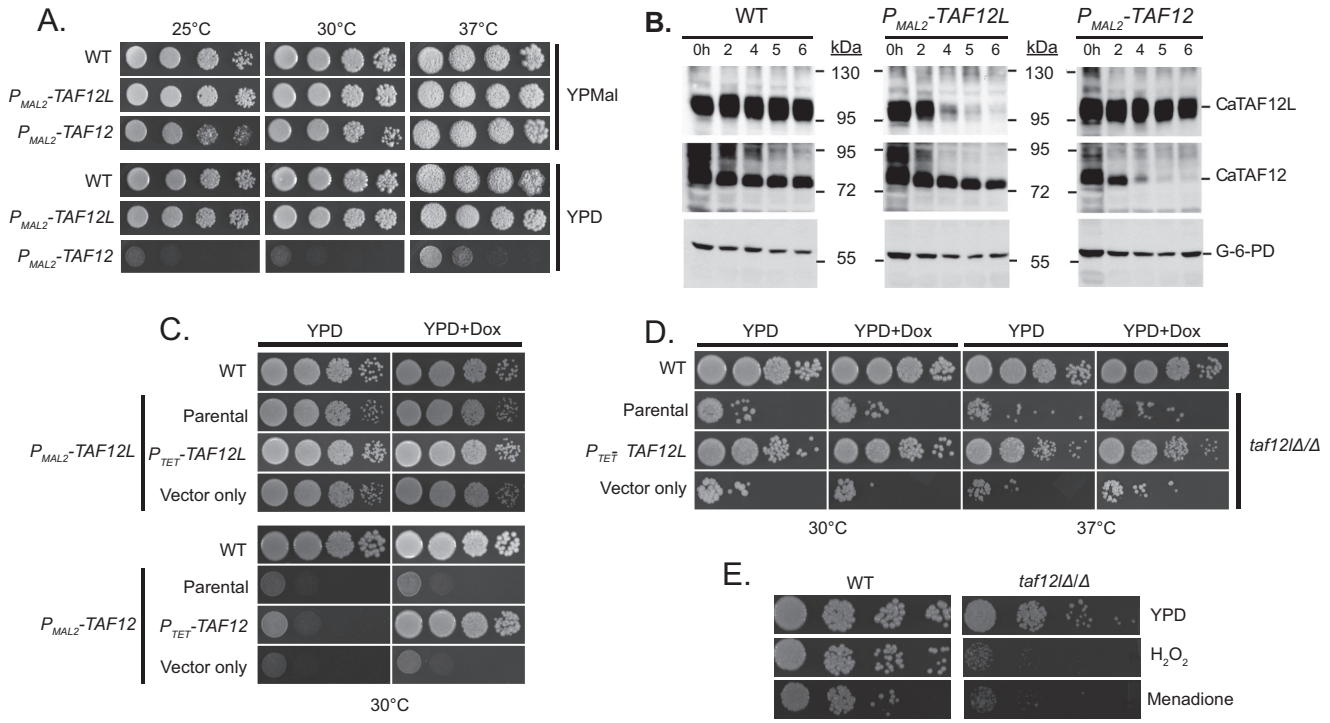


Figure 3. CaTAF12 but not *CaTAF12L* is essential for growth of *C. albicans*. *A–C*, conditional depletion of CaTAF12L and CaTAF12. *A*, the strains bearing glucose-repressible *CaTAF12L* (ISC11) or *CaTAF12* (ISC12) alleles or the wild type (SN95) as control were cultured in YP maltose (promoter-on), serial dilutions were spotted on YPMal or YPD (promoter-off) plates, and growth was monitored for 2 days. *B*, CaTAF12L and CaTAF12 protein levels were depleted upon promoter shut-off. Wild type, P_{MAL2}-*CaTAF12L*, and P_{MAL2}-*CaTAF12* strains were pre-grown in YPM and cultured in YPD, and cells were harvested. Whole-cell extracts from indicated time points were immunoblotted and probed with polyclonal rabbit anti-CaTAF12L, anti-CaTAF12, or the control anti-glucose 6-phosphate dehydrogenase (*G-6-PD*) antibodies. The positions of molecular mass markers are indicated in kDa. *C*, growth defects of CaTAF12L- and CaTAF12-depleted strains were rescued by *CaTAF12L* and *CaTAF12*, respectively. The strains WT (SN95), the parental strains bearing *PMAL2-CaTAF12L* (ISC11) or *PMAL2-CaTAF12* (ISC12) alleles, and the complemented strains P_{TET}-*CaTAF12L* (SDC13) and P_{TET}-*CaTAF12* (SDC16) or the vector-only control strains (SDC3 and SDC6) were grown overnight in the YPMal medium, and 10-fold serial dilutions were spotted on either YPD or YPD+doxycycline (*YPD+Dox*) plates, and then plates were incubated at 30 °C for 2 days. *D*, *C. albicans taf12*Δ/Δ null mutant is viable. The WT (SN95), *taf12*Δ/Δ mutant (parental; ISC36), and isogenic derivative strains bearing either P_{TET}-*CaTAF12L* (SDC17) or vector alone (SDC12) were cultured in YPD, serial dilutions were spotted onto YPD or YPD+Dox plates, and growth was monitored at 30 and 37 °C. *E*, *CaTAF12L* is required for oxidative stress resistance. *C. albicans* WT strain SN95 and *taf12*Δ/Δ null mutant (ISC36) were cultured in YPD, serial dilutions were spotted onto YPD or YPD plus 15 mM H_2O_2 or 60 μM menadione, and growth was monitored at 30 °C.

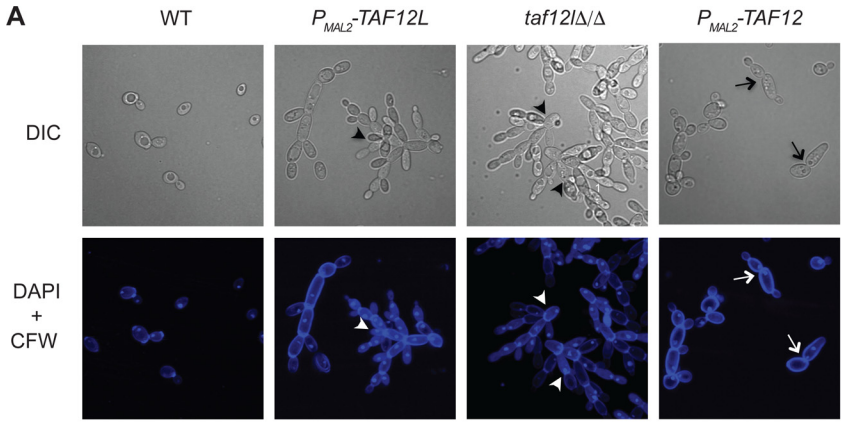
ination of growth under oxidative stress conditions imposed by H_2O_2 and menadione caused severe growth impairment of the mutant (Fig. 3*E*). Thus *CaTAF12L* is non-essential for vegetative growth but required for oxidative stress resistance in *C. albicans*.

The CaTAF12L- and CaTAF12-depleted strains showed distinct cellular morphologies (Fig. 4). Depletion of CaTAF12L, both in the promoter shut-off mutant ISC11 (P_{MAL2}-*CaTAF12L*) as well as in the *taf12l*Δ/Δ null mutant ISC36, led to pseudohyphal morphology comprising long, extensive chains of cells, each bearing a nucleus, indicating a cell separation defect (Fig. 4, *A* and *B*). CaTAF12 depletion, however, led to cells exhibiting unipolar buds or bipolar axial buds, or with small germ tube-like extensions beyond which the cells do not grow further (Fig. 4, *A* and *B*). Moreover, the CaTAF12L-depleted mutant showed reduced colony filamentation in different pseudohyphae-inducing solid media, *viz.* yeast carbon base (YCB) and synthetic low-ammonia dextrose media (SLAD), as well as attenuated hyphal colonies in serum-containing medium (supplemental Fig. S1). The *C. albicans taf12l*Δ/Δ null mutant, like the CaTAF12L-depleted mutant, formed rough colony morphology in all media tested, as well as deficient filamentation and agar invasion (supplemental Fig. S1). Together these results indicate that the *CaTAF12L* and *CaTAF12* genes make distinct contributions to *C. albicans* growth.

Discussion

TAFs are required for transcription of a wide array of genes. In this study, we investigated the role of two *TAF12* variants encoded by the paralogous genes orf19.470 (*CaTAF12L*) and orf19.6820 (*CaTAF12*) in *C. albicans*. Both the TAF12 variants contain the histone H2B-like histone-fold domain, shown previously to be essential for TAF12 function in yeast (30). Among all the fungal genome sequences examined, only eight diploid *Candida* clade genomes encoded the two TAF12 variants (Fig. 1*D*). Although orf19.6820/CaTAF12 coimmunoprecipitated with TBP, we found no specific enrichment of orf19.470/*CaTAF12L* with TBP, and they were accordingly named TAF12 and TAF12L as per the convention for TAF nomenclature (4). Our biochemical and genetic analyses revealed that *CaTAF12L* and *CaTAF12* are physically and functionally specialized to regulate gene expression by uniquely associating with the SAGA and the TFIID complexes, respectively.

In multicellular organisms, several paralogous TAF genes are tissue-restricted in their expression. Consequently, alternative TFIID and SAGA complexes are formed that function in a tis-



B

CaTAF12L depletion cellular morphology

	1	2	3	4	5	6	
	Normal	Elongated, 1-2 cells	3-4 cells	5-6 cells	6-10 cells	>10 cells	
P_{MAL2}-TAF12L	138 (33.7%)	72 (17.6%)	69 (16.9%)	42 (10.3%)	44 (10.8%)	44 (10.8%)	n=409
taf12lΔ/Δ	0 (0%)	84 (39.4%)	13 (6.1%)	13 (6.1%)	34 (16.0%)	69 (32.4%)	n=213

CaTAF12 depletion cellular morphology

	1	2	3	4	5	
	normal	unipolar axial, small chains	bipolar axial, small chains	Enlarged, 5-10 cells	small germ tube-like	
P_{MAL2}-TAF12	241 (58.6%)	44 (10.7%)	65 (15.8%)	18 (4.4%)	43 (10.3%)	n=411

Figure 4. The *taf12l* and *taf12* mutants have distinct cellular morphologies. *A*, strains SN95 (WT), ISC11 (P_{MAL2}-*CaTAF12L*), ISC36 (*taf12l*Δ/Δ), or ISC12 (P_{MAL2}-*CaTAF12*) were cultured overnight in YPM, diluted to fresh YPD, cultured for 6 h at 30 °C, and then fixed and stained with DAPI and CFW. Top row images were acquired by differential interference contrast (*DIC*) microscopy, and the bottom DAPI+CFW images were acquired by fluorescence microscopy, and images were digitally zoomed (2×). The altered cellular morphologies showing chains of cells are indicated by *arrowheads* in *taf12l*Δ/Δ and P_{MAL2}-*CaTAF12L*, and the bipolar axial budding or short evaginations in P_{MAL2}-*CaTAF12* are indicated by *arrows*. *B*, summary of the cellular morphology of *C. albicans* depleted for TAF12L or TAF12. *Top*, cell counts from the different categories of cellular morphologies of *C. albicans* strains. *Bottom*, summary of cell counts from different cellular morphologies upon TAF12 depletion.

sue- and cell type-specific manner (45, 46). In this respect, we found that the *C. albicans* TAF12L and CaTAF12 variants are constitutively expressed in vegetatively growing *C. albicans* cells (Fig. 2). Moreover, in yeast cells, TAF12 can directly heterodimerize with either TAF4 (47) or Ada1 (38). Therefore it is conceivable that CaTAF12L could associate with the SAGA complex through heterodimerization with Ada1 and CaTAF12 with the TFIID complex by heterodimerization with TAF4. Moreover, our *S. cerevisiae* complementation experiments indicated that CaTAF12L and CaTAF12 histone-fold domains are functionally distinct because only CaTAF12L HFD provided partial complementation in yeast.

How might the unique specialization of the two TAF12 variants for TFIID and SAGA influence cellular function? Past studies have shown that TAF12 is involved in interactions of the SAGA complex with the yeast transcriptional activators Gal4 and Gcn4 (31, 48–50). Interestingly, Gal4 activation domain cross-links to TAF12 only in the context of SAGA and not TFIID, indicating a complex-specific preferential role for TAF12 in activator interactions. Furthermore, the Gcn4 activation domain specifically cross-links to the amino-terminal 1–277 residues in the yeast TAF12 (50). However, *C. albicans* CaTAF12L and CaTAF12 are completely unrelated outside the HF domain, suggesting that a putative transcriptional activator is likely to be highly selective for CaTAF12L and CaTAF12 in *C. albicans*. Moreover, the *Drosophila* TAF12 along with TAF4, TAF5, TAF6, and TAF9 constitute a stable core of the *Drosophila* TFIID complex to which other TAFs and TBP associate (51). Our finding that CaTAF12 interacts with TFIID subunits and is essential for *C. albicans* growth is supportive of its central role in stabilizing the TFIID.

Although CaTAF12L and CaTAF12 associate specifically with separate complexes, our microscopy data showed that both the genes are required for optimal cell cycle progression but that they act in distinct steps. Although CaTAF12L mutation led to chains of cells that failed to undergo cell separation, CaTAF12 mutation caused arrest of cells either with small bud or with a slight evagination. Our finding that CaTAF12L is essential for oxidative stress resistance is supported by a previous report that the SAGA subunit Ada2 is required for oxida-

tive stress resistance in *C. albicans* (52). Thus CaTAF12L, being non-essential for vegetative growth, paradoxically has an essential role in modulating stress resistance in *C. albicans*. The evolution of two TAF12 variants in *C. albicans* and the closely related seven diploid pathogenic *Candida* genomes, although this could be a relic of speciation, nevertheless seems to provide this group of fungal pathogens a means to regulate cell growth in a distinct manner. We anticipate that our findings would lead to future studies to dissect the non-essential TAF12L to better understand the role of shared TAFs in supporting genome-wide transcription.

Experimental procedures

Growth conditions, strains, and plasmids

C. albicans strains SN87, SN95, and SN152 (53) were used as parental strains for all strains derived in this study. *S. cerevisiae* strains were derived from BY4743. Both *S. cerevisiae* and *C. albicans* strains were cultured in yeast extract-peptone (YP)-rich medium (54) for genetic analyses and protein extraction. All media were supplemented with 20 g/liter of either glucose or maltose as indicated.

The genome sequences and coordinates of genes of our interest were obtained from the Candida Genome Database (CGD). *C. albicans* SC5314 genomic DNA was used as template for all plasmid constructs, unless indicated otherwise, and all constructs were verified by restriction digestion and sequencing. Details of strain and plasmid constructions are described in the supplemental text. A list of *Candida albicans* genes reported in this study is provided in supplemental Table S1. Plasmids and strains used in this study are listed in supplemental Tables S2 and S3, and the list of oligonucleotides is provided in supplemental Table S4.

Immunoblotting

C. albicans strains were grown overnight (~18–20 h) in YP medium containing maltose and inoculated to a starting A_{600} of 0.15 (SN95 and P_{MAL2}-*CaTAF12L*) or 0.25 (P_{MAL2}-*CaTAF12*) into fresh YP medium containing glucose. Cultures were grown at 30 °C, and cells were harvested at different time points by centrifugation at 2450 × *g* for 5 min. Protein extracts were prepared and quantitated as described (55). About 50 μg of protein extracts were resolved on 8% SDS-PAGE gel and immunoblotted as described (55). The blots were probed with anti-CaTAF12L (1:1000) and anti-CaTAF12 (1:1000) rabbit polyclonal antibodies, with glucose 6-phosphate antibody (1:1000) as a control (Sigma). All blots were visualized using ECL Plus chemiluminescent system (GE Healthcare). The details of construction and purification of recombinant CaTAF12L and CaTAF12, as well as rabbit anti-CaTAF12L and anti-CaTAF12 polyclonal antibody production (see supplemental text).

Coimmunoprecipitation

The indicated *C. albicans* strains were cultured in YP medium containing glucose at 30 °C for 5 h, and whole-cell extracts were prepared as described previously (55). About 1 mg each of the whole-cell extracts was mixed with either 5 μl of IgG-Sepharose beads for TAP IPs or 10 μl of anti-FLAG M2-agarose beads (Sigma) for FLAG IPs and then incubated on a rotating platform at 4 °C for 2 h. After IP, the beads were collected, washed, and boiled in 1× SDS-PAGE sample buffer. 2-fold serial dilutions of the immunoprecipitated samples and 100 μg of input cell extracts were separated by 8% SDS-PAGE, and then immunoblotted as described (55). The following antibodies were used at 1:1000 dilution to probe the immunoblots: rabbit IgG Fc fragment (Jackson ImmunoResearch Laboratories), anti-HA (12CA5, Roche Applied Science), mouse polyclonal anti-TAF4 and anti-Ada1, and rabbit polyclonal anti-CaTAF12L and anti-CaTAF12 antibodies. Anti-FLAG antibody was used at 1:2500. All blots were visualized using ECL Plus chemiluminescent system (GE Healthcare).

Microscopy

C. albicans strains were cultured in the indicated medium, and then cells were harvested, washed with sterile 1× PBS, and fixed in 4% formaldehyde for 1–2 h at room temperature on a rotator. The fixed cells were again washed several times with 1× PBS. For cell imaging, an 0.1–0.2 A_{600} equivalent of the fixed cells was stained with a 1:20 dilution of 0.1 mg/ml DAPI for 20–30 min in the dark. Cells were pelleted, and a 100-fold dilution of 1 mg/ml calcofluor white (CFW) was added, incubated for 10 min in the dark, washed four times with 1× PBS, and mounted on slides, and images were acquired in a confocal microscope (Olympus FluoView FV1000) using 100× objective (oil immersion).

Author contributions—I. S., P. P., S. S., and K. N. designed research; I. S., P. P., and S. S. performed experiments; I. S., P. P., and K. N. analyzed data; I. S. drafted the manuscript, and K. N. edited and completed the manuscript. All authors read and approved the manuscript.

Acknowledgments—We are grateful to K. Ganesan for providing the promoter-replacement constructs prior to their publication. We also thank Suzanne Noble, Alexander Johnson, and Joachim Morschhauser for other plasmids and strains, and Shambhu Kumar for strain construction of FLAG-tagged strains. We thank Jerry Workman, Swaminathan Venkatesh, and members of the Workman laboratory for helpful discussions, and members of the Natarajan laboratory for discussions and comments on the manuscript. We acknowledge equipment use at the Central Instrumentation Facility (CIF), School of Life Sciences and the Confocal Microscopy Facility at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU).

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