Isolation and characterization of the *Candida albicans* gene for mRNA 5′-triphosphatase: association of mRNA 5′-triphosphatase and mRNA 5′-guanylyltransferase activities is essential for the function of mRNA 5′-capping enzyme in vivo

Toshiko Yamada-Okabe[a], Toshiyuki Mio[b], Mitsuaki Matsui[a], Yuji Kashima[a], Mikio Arisawa[b], Hisafumi Yamada-Okabe[b,*]

[a] *Department of Hygiene, Yokohama City University, 3-9 Fukuura, Kanazawa, Yokohama 236-0004, Japan*
[b] *Department of Mycology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*

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Abstract The amino acid sequence of the *Saccharomyces cerevisiae* mRNA 5′-triphosphatase (TPase) diverges from those of higher eukaryotes. In order to confirm the sequence divergence of TPases in lower and higher eukaryotes, the *Candida albicans* gene for TPase was identified and characterized. This gene designated *CaCET1* (*C. albicans* mRNA 5′-capping enzyme triphosphatase 1) has an open reading frame of 1.5 kb, which can encode a 59-kDa protein. Although the N-terminal one-fifth of *S. cerevisiae* TPase (ScCet1p) is missing in CaCet1p, CaCet1p shares significant sequence similarity with ScCet1p over the entire region of the protein; the recombinant CaCet1p, which was expressed as a fusion protein with glutathione *S*-transferase (GST), displayed TPase activity in vitro. *CaCET1* rescued *CET1*-deficient *S. cerevisiae* cells when expressed under the control of the *ADH1* promoter, whereas the human capping enzyme derivatives that are active for TPase activity but defective in mRNA 5′-guanylyltransferase (GTase) activity did not. Yeast two-hybrid analysis revealed that *C. albicans* Cet1p can bind to the *S. cerevisiae* GTase in addition to its own partner, the *C. albicans* GTase. In contrast, neither the full-length human capping enzyme nor its TPase domain interacted with the yeast GTase. These results indicate that the failure of the human TPase activity to complement an *S. cerevisiae cet1*Δ null mutation is attributable, at least in part, to the inability of the human capping enzyme to associate with the yeast GTase, and that the physical association of GTase and TPase is essential for the function of the capping enzyme in vivo.

Key words: mRNA 5′-capping; mRNA 5′-triphosphatase; *Candida albicans*; Cloning; Expression; Physical association; Yeast two hybrid

1. Introduction

During transcription by RNA polymerase II, a cap structure is formed on the 5′-termini of most nascent nuclear pre-mRNAs. Capping of mRNA has been shown to be important for the stabilization [1–3], processing [4–9], nuclear export [10], and efficient translation of mRNA (see [11,12] for reviews). Capping involves at least three enzymes called mRNA 5′-triphosphatase (TPase), mRNA 5′-guanylyltransferase (GTase), and cap methyltransferase (MTase) (see [13,14] for reviews). TPase converts the 5′-triphosphate end of a nascent RNA chain into a diphosphate end, and GTase transfers the GMP moiety of GTP to the newly produced 5′-diphosphate end of RNA to form a blocking structure. Thereafter, MTase attaches a methyl group to the 7 position of the terminal guanosine of RNA. As GTase and TPase are essential for the synthesis of the core cap structure, they are called mRNA 5′-capping enzymes (termed capping enzyme in this study).

Purification of the *Saccharomyces cerevisiae* capping enzyme demonstrated that the enzyme consists of two subunits, α and β. GTase activity resides in the 52-kDa α subunit, which is encoded by an essential gene, *CEG1* [15], and TPase activity is intrinsic to the 80-kDa β subunit, which is specified by another essential gene, *CET1* [16]. The GTase genes have been identified also in *Schizosaccharomyces pombe* (*PCE1*) [17], *Candida albicans* (*CGT1*) [18], and chlorella virus [19], and all these products have been shown to possess only GTase activity. Moreover, the crystal structure of chlorella virus GTase was solved in complex with GTP. The enzyme contains two domains forming a deep and narrow cleft between them, whose size may fit the substrate RNA. Moreover, manganese ions induce a big conformational change of the enzyme, which leads to the hydrolysis of β and γ phosphoryl groups of the bound GTP [20].

Recently, cDNAs for capping enzyme were cloned in human, mouse and worm, and it became evident that the capping enzymes of higher eukaryotes consist of a single polypeptide that harbors both GTase and TPase activities. It was also demonstrated that in higher eukaryotic capping enzymes the N-terminal part is responsible for TPase activity and the C-terminal part is essential for GTase activity [21–25]. In addition, capping enzyme is associated with the elongating RNA polymerase II but not with resting RNA polymerase II, which accounts for the capping specificity to the transcripts by RNA polymerase II [21,26]. Interestingly, higher eukaryotic capping enzymes harbor an amino acid sequence motif that is characteristic of the active site of protein tyrosine phosphatase families within the N-terminal TPase domains [21–25], but this motif was not present in the *S. cerevisiae* Cet1p [16]. Furthermore, although active as TPase in vitro, the human capping enzyme, which is defective in GTase activity, failed to complement an *S. cerevisiae cet1*Δ null mutation [24]. All these facts suggest a different phylogeny for the TPase enzymes in yeast and higher eukaryotes.

*Corresponding author. Fax: (81) (467) 46-5320.
E-mail: hisafumi.okabe@roche.com

The GenBank/EMBL/DDBJ accession number for the nucleotide sequence of *CaCET1* is AB016242.

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ScCet1p: MSYTDNPPQTKRALSLDDLVNHDENEKVKLQKLSEAANGSRPFAENLESDINQTETGQAA : 60
                                                                    .
CaCet1p:                                                        MNVGS :  5

ScCet1p: PIDNYKESTGHGS-HSQKPKSRKSSNDDEETDTDDEMGASGEINFDSEMDFDYDKQHRNL :119
          ...   |.|. . .    |  ||     .    .    . .|          |    .
CaCet1p: ILNDDPSSSGNANGNDDNTKIIKSPTAYHKPSVHERHSITSMLNDTPSDSTPTKKPEPTI : 65

ScCet1p: LSNGSPP----MNDGSDANAKLEKPSDDSI---HQNSKSDEEQRIPKQGNEGNIASNYIT :172
               |    .   | |.     |   |      ..| .     | |. .  ||   |
CaCet1p: SPEFRKPSISSLTSPSVAHKPPPLPPSSSSVGSSEHSSARSSPAITKRNSIANIIDAYEE :125

ScCet1p: QVPLQKQKQTEKKIAGNAVGSVVKKEEEANAA--VDNIFEEKATLQSKK------NNIKR :224
            ..|       |    | | ||  |    |. . .    . ||       ..
CaCet1p: PATKTEKKAELNSPKINQSTPVPKLEEHENDTNKVEKVVDSAPEPKPKKEPQPVFDDQDD :185

ScCet1p: DLEVLNEISASSKPSKYKNVPIWAQKWKPTIKALQSINVKD------------------- :265
         ||  . ..| || .|.  |||||.| |  .  .  || |
CaCet1p: DLTKIKKLKQSKKPRRYETPPIWAQRWVPPNRQKEETNVDDGNEAITRLSEKPVFDYTTT :245

ScCet1p: --LKIDPSFLNIIPDDDLTKSVQDWVYATIYSIAPELRSFIELEMKFGVIIDAKGPDRVN :323
           . .. |   .||   .|. . .||||   ..  . .  .|||.||| ||| .  .|..
CaCet1p: RSVDLECSITGMIPPSSITRKIAEWVYANFSNVEEKSKRNVELELKFGKIIDKRSGNRID :305

ScCet1p: PPVSSQCVFTE-LDAHLTPNIDASLFKELSKYIRGIS---EVTENTGKFSIIESQTRDSV :379
           | ..|.||.          ..   .||..|..  ..  .   ||  .||   ||
CaCet1p: LNVVTECIFTDHSSVFFDMQVEEVAWKEITKFLDELEKSFQEGKKGRKFKTLESDNTDSF :365

ScCet1p: YRVGLSTQRPRFLRMSTD-IKTGRVGQFIEKRHVAQLLLYSPKDSYDVKISLNLELPVPD :438
         |..|   . |. .|.. | . .       |.|  || | ...|   .|...|..||.|||
CaCet1p: YQLGRKGEHPKRIRVTKDNLLSPPRLVAIQKERVADLYIHNPGSLFDLRLSMSLEIPVPQ :425

ScCet1p: NDPPEKYKSQSPISERTKDRVSYIHNDSCTRIDITKVENHNQNSKSRQSETTHEVELEIN :498
          .         |   | | |.|| |  . |. |.|.|  .       ..|  .|||||
CaCet1p: GNIESIITKNKPEMVREKKRISYTHPPTITKFDLTRVIGN-------KTEDKYEVELEAG :478

ScCet1p: TPALLNAFDNITN--DSKEYASLIRTFLNNGTIIRRKLSSLSYEIFEGSKKVM        :549
            .  | | |    |.     ||  ||||   .  .|. .
CaCet1p: VMEIFAAIDKIQKGVDNLRLEELIEVFLNNARTLNNRLNKIC                    :520
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Fig. 1. Comparison of the amino acid sequences of *C. albicans* and *S. cerevisiae* Cet1 proteins. The amino acid sequence of *C. albicans* TPase (CaCet1p) is compared with that of *S. cerevisiae* TPase (ScCet1p). Identical and homologous amino acids in these two proteins are marked by bars and dots, respectively. Amino acid sequences were aligned using the BLAST and FASTA programs.

To confirm the phylogenic divergence of TPase in lower and higher eukaryotes, we isolated the TPase gene from the pathogenic fungus *C. albicans*. The expected product of the *C. albicans* TPase is highly related to the *S. cerevisiae* TPase in most of the regions, but it shares no sequence homology with the TPase domains of the higher eukaryotic capping enzymes. The *C. albicans* TPase but not the TPase activity from the human capping enzyme rescued the TPase-deficient *S. cerevisiae* cells. Further, the *C. albicans* TPase was able to associate with the *S. cerevisiae* GTase, while the human TPase domain and the full-length human capping enzyme were not. Thus, the association of GTase and TPase activities seems to be essential for the function of the capping enzyme in vivo.

2. Materials and methods

2.1. Screening of a C. albicans DNA library

The partial sequence of *C. albicans CET1* (*CaCET1*) was found in the *C. albicans* database (web site, http://alces.med.umn.edu/bin/genelist?Lcet1). Using the nucleotide sequence of this clone, we amplified a 450-bp DNA fragment of *CaCET1* from the genomic DNA of *C. albicans* strain IFO1060 by PCR and used it as a probe for cloning a full-length DNA. Primers used to amplify the probe DNA were 5′-ACTCTTGAATCTGATAATACTGATAGTTTC-3′ and 5′-GACCCTAGTCAAGTCAATTTTGGTAATGGT-3′. Hybridization and washing of the filters were carried out under stringent conditions (20 mM sodium phosphate (pH 7.2), 5×SSC (1×SSC contains 150 mM NaCl and 15 mM sodium citrate), 5×Denhardt's solution, 0.1% (w/v) SDS, 50% (v/v) formamide at 42°C for hybridization; 0.1×SSC and 0.1% SDS at 50°C for washing), and plasmid DNA was extracted from bacterial clones that were strongly hybridized with the probe DNA. Radiolabelling of the probe DNA was performed by the random priming method with [α-^{32}P]dCTP [27], and DNA sequencing was carried out as described elsewhere [27]. Construction of the *C. albicans* genomic DNA library has already been described [18].

2.2. Expression and purification of the recombinant TPase

The coding regions of the *S. cerevisiae* and *C. albicans CET1* genes were cloned at the *Bam*HI and *Sma*I sites of pGEX2T, respectively [28]. The resulting plasmids were transfected into *Escherichia coli* JM109, and they were induced by IPTG to express *S. cerevisiae* and *C. albicans* Cet1 proteins as fusion proteins with GST [28]. At 4 h after the addition of IPTG, the bacterial cells were harvested, suspended in a buffer containing 20 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 50 mM NaCl, 10 mM β-mercaptoethanol, 10% (v/v) glycerol, 0.05% (v/v) NP40, and 1 mM PMSF, and lysed by sonication. After the cell debris was removed by centrifugation at 15 000×*g* at 4°C for 30 min, the recombinant Cet1 protein in the supernatant was purified by glutathione Sepharose CL-4B column chromatography. Preparation of the recombinant fusion protein of GST and the *C. albicans* GTase (CaCgt1p) has already been described [18].

2.3. Assays for TPase

The assays for TPase were carried out in a buffer containing 50 mM Tris-HCl (pH 7.9) and 2 mM dithiothreitol, [γ-^{32}P]ATP-terminated RNA, and the indicated amounts of the purified fusion proteins at 30°C for 30 min [29]. After removing the beads by a brief centrifugation, the reaction mixture was analyzed by TLC using polyethylene-

imine cellulose plates, and the spots were visualized by an image analyzer (Fuji BAS 2000). The [γ-^{32}P]ATP-terminated RNA was prepared by incubating 50 μg of heat-denatured calf thymus DNA in a buffer containing 100 mM Tris-HCl (pH 7.9), 2 mM dithiothreitol, 5 mM $Mg(OAc)_2$, 2 mM $MnCl_2$, 1 mM [γ-^{32}P]ATP (1000–5000 cpm/pmol), and 1 unit of *E. coli* RNA polymerase at 37°C for 90 min, and purified by Sephadex G-25 column chromatography, as described previously [29].

The in vitro cap formation assay was performed in a buffer containing 10 mM Tris-HCl (pH 7.5), 3 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mM EDTA, 20% (v/v) glycerol, 2.4 mM [α-^{32}P]GTP (400 Ci/mmol), 0.1 μg of the purified GST-CaCet1p, 0.1 μg of the purified GST-CaCgt1p, and approximately 50 pmol pppA-terminated RNA at 30°C for 60 min. After RNA was digested with nuclease P1 and calf intestine alkaline phosphatase, the cap structure was separated on DE81 paper by paper electrophoresis and visualized by image analyzer (Fuji BAS 2000) [29].

2.4. Yeast two-hybrid analysis

The entire regions of the ORFs of *ScCEG1* and *CaCGT1* were cloned at the *Sal*I site of pGBT9, generating pGBT9-ScCEG1 and pGBT9-CaCGT1. Thus, ScCeg1p and CaCgt1p would be expressed as fusion proteins with the DNA binding domain of Gal4p. The ORFs of the indicated yeast and human capping enzyme genes were also cloned at the *Sal*I site of pGAD424 (Clontech) to express these products as fusion proteins with the transactivation domain of Gal4p. Then, the resulting plasmids were transformed into *S. cerevisiae* strain HF7c (*MATa ura3-52 his3-200 lys2-801 ade2-101 trp1-901 leu2-3 112 gal4-542 gal80-538 LYS2::GAL1-HIS3 URA3::(GAL4 17-MERS)$_3$-CYC1-LACZ*), where *HIS3* gene expression was driven by the DNA-binding and transactivation domains of Gal4p [30]. After the transformation of HF7c, the leu$^+$ trp$^+$ transformants were collected and tested for their ability to grow in the absence of histidine.

2.5. Generation of yeast strains

Generation of a *cet1*Δ (*MATa ade2 ura3 leu2 his3 trp1 cet1*Δ::*LEU2 CET1-URA3*) null mutant strain, *cet1*Δ::*LEU2*, in which the endogenous *CET1* was disrupted by *LEU2*, but where episomal copies of *CET1* cloned in YEp24 were maintained, has already been described in a previous paper [24]. The ORFs of the *S. cerevisiae* and *C. albicans CET1* genes and the human capping enzyme cDNAs were cloned between the *Hin*dIII and *Pst*I sites (between the *ADH1* promoter and terminator) of pGBT9 carrying *TRP1* as a selection marker (Clontech), and the resulting plasmids were transformed into the *cet1*Δ::*LEU2* cells. Because the above *cet1*Δ::*LEU2* cells cannot grow in the presence of 5-fluoroorotic acid (5-FOA), the ability of *C. albicans CET1* and the human capping enzyme cDNAs to complement a *S. cerevisiae cet1*Δ null mutation was examined by culturing them on an agar plate containing 5-FOA.

3. Results

3.1. Cloning of the C. albicans TPase gene

A partial sequence of the *C. albicans CET1* (*CaCET1*) gene is available in the *C. albicans* database (http://alces.med.umn.edu/bin/genelist?Lcet1). The 0.5-kb DNA fragment containing *CaCET1* was amplified and used as a probe for screening a *C. albicans* genomic DNA library. Plasmid DNA was extracted from the clones that were strongly hybridized with the probe DNA and were analyzed by restriction enzyme mapping and Southern blotting. The insert DNA of all the plasmids contained overlapping sequences of the *C. albicans* genome. Complete nucleotide sequencing of the shortest insert among the isolated clones revealed that it contained an ORF of 1.5 kb, which can encode a protein of 59 kDa. Although the N-terminal one-fifth of ScCet1p is missing in the expected product of CaCet1p, CaCet1p shares significant sequence similarity with ScCet1p over the entire region of the protein (24.8% identity and 45.4% similarity) (Fig. 1). As reported in the previous paper, there was essentially no sequence homology between ScCet1p and the human capping enzyme, Hce1p [24,25]. We also aligned the amino acid sequences of CaCet1p and Hce1p using the FASTA and BLAST programs, but CaCet1p shows no sequence similarity to any part of Hce1p.

Next, we examined the enzyme activity of CaCet1p. CaCet1p was expressed as a fusion protein with GST and purified by affinity column chromatography (Fig. 2A). The purified GST-CaCet1 fusion protein efficiently released [^{32}P]Pi from the [γ-^{32}P]triphosphate-terminated RNA, while GST

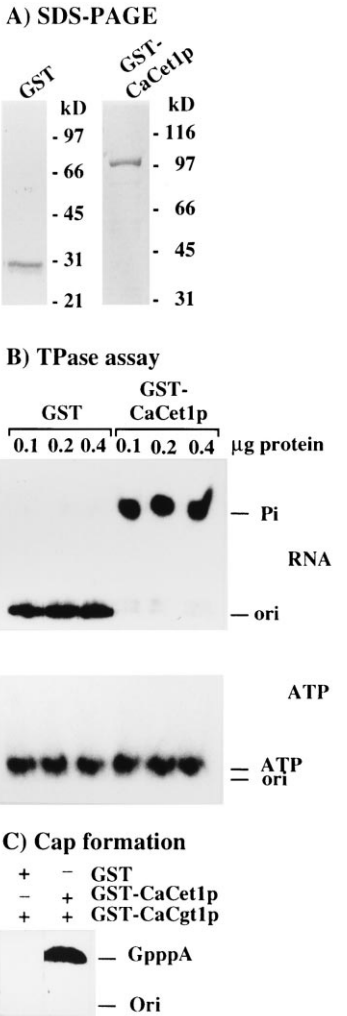

Fig. 2. Expression and enzyme activities of the *C. albicans* TPase. The *C. albicans* Cet1p was expressed in *E. coli* as a fusion with GST and purified by glutathione Sepharose affinity column chromatography. A: Approximately 0.1 μg of the purified GST and GST-CaCet1p fusion protein were separated on a 12.5% (for GST) and 10% (for GST-CaCet1p) SDS-polyacrylamide gel, respectively, and were stained with Coomassie brilliant blue. B: The indicated amounts of the purified GST and GST-CaCet1p were incubated with γ-^{32}P-terminated RNA (upper panel) or [γ-^{32}P]ATP (lower panel), and the released inorganic phosphates were detected by TLC. The positions of inorganic phosphate (Pi), ATP (ATP) and origin (ori) on the TLC plates are indicated. C: Approximately 0.1 μg of the purified GST and GST-CaCet1p fusion protein were incubated with 5′-triphosphorylated RNA, [α-^{32}P]GTP, and the recombinant *C. albicans* GTase (GST-Cgt1p). The cap structure formed at the 5′-end of the RNA was separated on DE81 paper. The positions of the formed cap structure (GpppA) and origin (ori) on the DE81 paper are indicated.

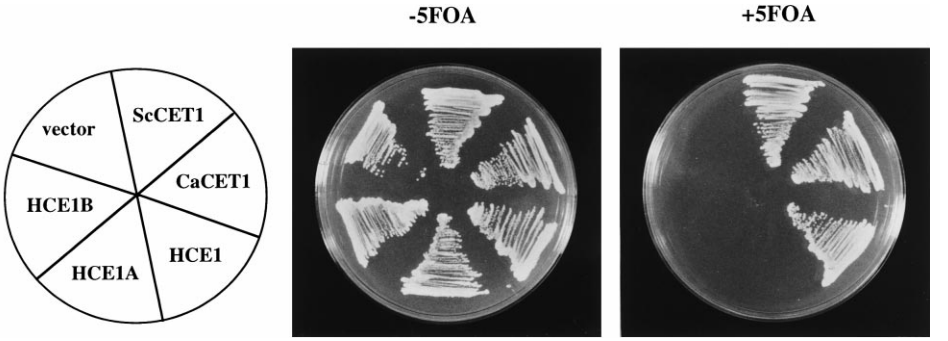

Fig. 3. Functional complementation of a *S. cerevisiae cet1*Δ null mutation by the *C. albicans CET1* gene. The *S. cereviaise cet1*Δ::*LEU2* cells that harbored *ScCET1* in YEp24 were further transformed with pGBT9 bearing *ScET1*, *CaCET1*, *HCE1*, *HCE1A*, or *HCE1B*. Transfectants were spread on agar plates with (+) or without (−) 5-FOA, and incubated at 30°C for 3 days.

alone did not. Removal of the γ phosphate at the 5′-terminus of RNA would be rather specific to RNA, because it did not produce [^{32}P]Pi when incubated with [γ-^{32}P]ATP (Fig. 2B). We also performed a cap formation assay to determine whether CaCet1p created a diphosphate end of RNA. This was based on the fact that cap formation by the eukaryotic GTase requires an RNA with a 5′-diphosphate end as the substrate [31]. When the recombinant *C. albicans* GTase that was also expressed as a fusion protein with GST (GST-CaCgt1p) was included in the reaction mixture, GST-CaCet1p but not GST alone formed a cap structure on the 5′-triphosphate-terminated RNA (Fig. 2C), demonstrating that CaCet1p converted the 5′-triphosphate end of the substrate RNA to a diphosphate end. From these results, we conclude that *CaCET1* specifies an active mRNA 5′-triphosphatase of *C. albicans* with characteristics similar to those of *S. cerevisiae* Cet1p.

3.2. Complementation of an *S. cerevisiae ceg1Δ* null mutation by *C. albicans CET1* and interaction of CaCet1p with ScCeg1p

As mentioned before, the N-terminal one-fifth of ScCet1p is missing in CaCet1p, and CaCet1p has an insertion of 21 amino acids between positions 227 and 247. This prompted us to ask whether *CaCET1* functionally substitutes for *S. cerevisiae CET1*. The *S. cerevisiae cet1*Δ null mutant strain, *cet1*Δ::*LEU2*, was created by disrupting the endogenous *CET1* gene by the *LEU2* gene and introducing episomal copies of *CET1* in an *URA3*-containing plasmid (YEp24) [24]. Thus, the *cet1*Δ::*LEU2* grew in the absence of 5-FOA, but died in the presence of 5-FOA. Expression of *CaCET1* under the control of the *ADH1* promoter rescued the *cet1*Δ::*LEU2* cells even in the presence of 5-FOA (Fig. 3), demonstrating that *C. albicans* Cet1p functionally complements *S. cerevisiae* Cet1p.

In addition to *HCE1*, *HCE1A* and *HCE1B* have been identified as variants of *HCE1*. Hce1p possesses both TPase and GTase activities. Hce1a and Hce1b proteins, however, have deletions within the ORF and lack GTase activity [24]. As reported previously [24] and also shown in Fig. 3, we reproducibly observed that *HCE1* but not *HCE1A*, and *HCE1B* complemented an *S. cerevisiae cet1*Δ null mutation despite the fact that all of these products displayed TPase activity in vitro [24]. Because GTase and TPase activities always reside in one enzyme, the failure of Hce1a and Hce1b proteins to complement an *S. cerevisiae cet1*Δ null mutation may be due to the failure of complex formation between the yeast GTase and the human capping enzyme. Further, CaCet1p might bind to ScCeg1p to functionally substitute for ScCet1p in vivo. To address this possibility, yeast two-hybrid analysis was performed. HF7c cells harboring pGBT9-ScCEG1, HF7c-pGBT9-ScCEG1, grew in the absence of histidine, when they were further transfected with pGAD424-ScCET1 or pGAD424-CaCET1. In contrast, pGAD424-HCE1, pGAD424-HCE1A and pGAD424-HCE1B conferred no histidine prototrophy on HF7c-pGBT9-ScCEG1 (Fig. 4). We constructed pGAD424-HCE-TP, which harbored only the TPase domain of *HCE1*, to exclude the possibility that the GTase domain of Hce1p blocks the binding of the TPase domain of Hce1p and ScCeg1p, and found that it also failed to support the histidine-independent growth of HF7c-pGBT9-ScCEG1 (data not shown). The same results were obtained with the HF7c-pGBT9-CaCGT1 cells that carried *CaCGT1* instead of *ScCEG1* (Fig. 4). All these results indicate that the GTases and TPases of *S. cerevisiae* and *C. albicans* interact with each other to form a subunit structure of the enzyme, but they do not bind to the human enzyme.

Previously, it was demonstrated that the molecular mass of the *S. cerevisiae* capping enzyme was about 180 kDa [32]. Because the predicted molecular masses of ScCeg1p and ScCet1p are 52 kDa [15] and 62 kDa [16], respectively, the subunit structure of the *S. cerevisiae* capping enzyme may be

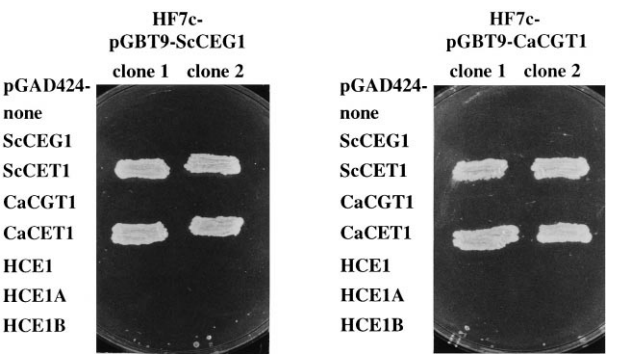

Fig. 4. Interspecies association of GTase and TPase of *S. cerevisiae* and *C. albicans*. Yeast HF7c cells bearing pGBT9-ScCEG1 (HF7c-pGBT9-ScCEG1, right panel) or pGBT9-CaCGT1 (HF7c-pGBT9-CaCGT1, left panel) were further transfected with the pGAD424 derivatives carrying the indicated genes. Two independent colonies from each transformation were collected and further cultured on histidine-depleted agar plates for 3 days.

α1β2 or α2β1. This prompted us to examine whether ScCeg1p or ScCet1p forms a homodimer; however, the yeast two-hybrid analysis did not indicate such GTase-GTase and TPase-TPase interactions (Fig. 4).

4. Discussion

We have identified the TPase gene from the pathogenic fungus, *C. albicans*. The predicted amino acid sequence of CaCet1p is related to that of ScCet1p, but it is quite different from those of the TPase domains of higher eukaryotic capping enzymes. Moreover, higher eukaryotic capping enzymes harbor the amino acid sequence motif characteristic of protein tyrosine phosphatase families within the TPase domain, but this motif was not found in ScCet1p and CaCet1p [21–25]. Thus, it is likely that the TPases of lower eukaryotes and higher eukaryotes are phylogenically divergent. Although the N-terminal one-fifth of the ScCet1p is missing in CaCet1p, the findings that the recombinant CaCet1p displayed TPase activity in vitro and that *CaCET1* functionally complemented an *S. cerevisiae cet1*Δ null mutation indicate that the N-terminal part of ScCet1p is not essential for the enzyme function. This is consistent with the report by Tsukamoto et al. [16] that the active site should reside within the C-terminal two-thirds of the protein. In fact, the sequence similarity between ScCet1p and CaCet1p is higher within the C-terminal two-thirds of the two proteins.

CaCET1 functionally complemented a *S. cerevisiae cet1*Δ null mutation, and CaCet1p interacted with ScCeg1p. *HCE1*, whose product has been shown to harbor both TPase and GTase activities in one polypeptide, also rescued the *CET1*-deficient *S. cerevisiae* cells, whereas *HCE1A* and *HCE1B*, whose products have been demonstrated to possess only TPase activity, failed to functionally substitute for the yeast TPase in vivo. Because the Hce1, Hce1a, and Hce1b proteins were unable to associate with the yeast GTases as judged by the yeast two-hybrid analysis, we concluded that the failure of *HCE1A* and *HCE1B* to complement the yeast *cet1*Δ null mutation was due at least in part to the inability of the human capping enzyme to bind to the yeast GTase, and that the association of TPase and GTase activities is essential for the function of capping enzyme in vivo. Tsukamoto et al. [16] demonstrated the interaction of *ScCEG1* and *ScCET1* proteins by the protein overlay assay using radiolabelled ScCeg1p. We also intended to confirm the interaction of the *C. albicans* and *S. cerevisiae* capping enzyme subunits by the overlay assay, but it was not successful when the GST-ScCeg1p and GST-CaCgt1p fusion proteins were used as probes.

As mentioned before, the possible subunit structure of the *S. cerevisiae* capping enzyme is α1β2 or α2β1, according to the molecular masses of the purified *S. cerevisiae* capping enzyme (180 kDa), ScCeg1p (52 kDa) and ScCet1p (62 kDa). The yeast two-hybrid analysis, however, did not suggest self-interaction of the yeast GTases and TPases. Thus, it seems that the *S. cerevisiae* GTase or TPase has two binding sites for its partner. Although the molecular mass of the native capping enzyme of *C. albicans* remains to be established, the subunit structure of the capping enzyme may be well conserved between the two species, because the TPases and GTases of *S. cerevisiae* and *C. albicans* are functionally complementable and interactive.

In the previous paper, it was demonstrated that in *S. cerevisiae* the capping enzyme that was purified from the zymolyase-treated yeast cells consisted of a 45-kDa α-subunit and a 39-kDa β-subunit. Because the purified capping enzyme possessed both GTase and TPase activities, the 39-kDa β-subunit would be an N-terminally truncated form of ScCet1p. In addition, the 39-kDa N-terminally truncated ScCet1p would have the ability to bind to GTase [33]. Thus, the C-terminal two-thirds of ScCet1p seem to be responsible both for TPase activity and for the interaction with GTase. Accordingly, the N-terminal part of the yeast Cet1p may serve as an additional binding site for GTase.

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