A Temperature-Regulated, Retrotransposon-Like Element from Candida albicans

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A repetitive element was isolated from the genome of Candida albicans. This repetitive element, which we designated alpha, was localized to a 500-bp fragment of genomic DNA. The alpha element was dispersed in the genome and varied in copy number and genomic location in the strains examined. Analyses of various loci containing the alpha element identified a locus containing a composite element. This composite element consisted of two direct repeats of the alpha element separated by approximately 5.5 kb of DNA, a structural arrangement similar to that of retrovirus-like transposable elements. The flanking alpha elements of the composite structure were 388 bp in length and were identical in sequence. They were bounded by the nucleotides 5'-TG...CA-3', which were part of a delimiting inverted repeat, a feature conserved in the long terminal repeats of retroviruses and retrovirus-like elements. As in retrovirus-like elements, the entire composite element, including the alpha elements, was transcribed into an approximately unit-length mRNA. The expression of this transcript was greatly increased when cells were grown at 25 versus 37°C. As has been found in many retrotransposons, the composite element was flanked by a 5-bp duplication and varied in both copy number and genomic location in various strains. We conclude that the composite element is a retrotransposon-like element, and we have designated this element Tca1. We suggest that Tca1 may be relevant to the genomic evolution of C. albicans and the pathogenic potential of the organism.

Candida albicans is a dimorphic, asexual fungus. Although typically found as a commensal organism of the human gut (44, 55), C. albicans is often the agent of opportunistic infections (44). These infections range in severity from mild mucocutaneous forms to systemic and disseminated forms, which are often fatal (44). The species C. albicans appears to consist of a large array of diverse strains (41). Strain variability has been demonstrated in many phenotypic and genotypic characteristics, some of which may be relevant to the virulence of C. albicans. These characteristics include adherence (18, 39), dimorphic ability (37), proteinase production (18, 60), switching of colony morphology (57, 58), and virulence in experimental animals (43). The basis of these phenotypic differences is unknown, but they presumably reflect strain-specific mutational alterations. C. albicans is known to harbor recessive heterogeneous mutations, the spectrum of these mutant alleles being strain variable (68, 69). The nature of these mutations is unknown. While chromosomal rearrangements recently have been suggested as a source of genetic variation (50), the genetic mechanisms contributing to the strain diversity of C. albicans have been little explored. Understanding these mechanisms could provide insight into the role of genomic diversity in the evolution of the host-pathogen interaction and the nature of virulence and pathogenesis in C. albicans.

Mobile genetic elements, because of their ability to alter genomic structure and gene expression, may be of particular importance in an asexual organism such as C. albicans. The genetic parallels between Candida spp. and Saccharomyces cerevisiae (29) suggest that the mobile elements of S. cerevisiae might provide a useful paradigm for C. albicans. Four retrotransposons, Ty1, Ty2, and Ty3 (reviewed in references 3 and 47) and the recently described Ty4 (35, 62), have been found for S. cerevisiae. As in retroviruses, these Ty elements are composite elements consisting of long terminal repeats (LTRs) approximately 340 bp in length and flanking an internal domain of roughly 5.5 kb. This internal domain encodes the functions required for retrotransposition, including reverse transcriptase, and shares a number of features conserved in retroviruses (21, 67). The LTRs of Ty1 and Ty2 are called delta elements, while the LTRs of Ty3 and Ty4 are referred to as sigma and tau elements, respectively. The LTRs are often found as solo elements divorced from the composite structure. These solo elements arise by homologous recombination between the flanking repeats of the retrotransposon, with the subsequent deletion of the intervening sequence (46). Consequently, solo LTRs, like Ty elements, are flanked by 5-bp direct repeats resulting from the target site duplication that occurs during integration (47).

Strain comparisons indicate variable copy numbers of the elements. Ty1 is present at 30 to 35 copies in laboratory strains, but only a few copies are present in natural isolates (3, 12, 47). Approximately 10 copies of Ty2 are present in laboratory strains, but Ty3 and Ty4 exist in only 1 to 4 copies per cell (7, 62). The solo LTRs are more numerous, with copy numbers of 100 or more of delta and approximately 20 each of sigma and tau (3).

Transposition of Ty elements occurs via an RNA intermediate (4). Transcription of the elements initiates in the 5'-LTR and terminates in the 3'-LTR, generating a full-length transcript with redundant termini (13). The rate of transposition is dependent on the level of transcription (8). Ty transcription responds to mating type control and is elevated in MATa or MATα cells relative to MATa/MATα diploids (14). Transcription is also elevated by UV light (40, 48) and DNA-damaging agents (40). Ty3 is additionally inducible by mating type pheromones (7).

Ty elements can have dramatic effects on gene expression. Integration of a Ty element into the promoter or coding
region of a gene can completely inactivate the gene (3). Conversely, integration into the promoter region can also result in constitutive activation of a gene or MAT-regulated expression of the adjacent gene (3). MAT regulation of adjacent gene expression is almost always associated with divergent transcription of the integrated Ty element (3). These effects are restricted to Ty1 and Ty2 elements. However, since Ty1 and Ty2 integration is not highly specific, many genes have been found to be influenced by integration of these elements (3). Ty3, which is integrated exclusively adjacent to tRNA genes (7, 21), has not been shown to affect adjacent gene expression. However, sigma-specific transcripts that respond to yeast mating type factors have been observed (7, 64). This observation implies that solo sigma elements can act as independent promoters, as has been observed for delta elements (7, 64).

In addition to the effects of Ty transposition per se, Ty elements may be passive participants in other types of genomic rearrangements. Homologous recombination between Ty elements integrated at different chromosomal loci can result in deletions, inversions, and translocations (6, 34, 46). Similarly, deletions and inversions due to recombination between Ty elements and solo delta elements or recombination between solo delta elements also have been observed (11, 49).

We report here the isolation of a previously unidentified dispersed repetitive element from *C. albicans*. This element exhibited several features conserved within the LTRs of retroviruses and retrotransposons and was found as part of a composite element that was structurally similar to retrotransposons. Expression of the composite element was strongly dependent on temperature. We suggest that this putative retrotransposon may be relevant to the genotypic diversity of *C. albicans*.

**MATERIALS AND METHODS**

**Strains and culture conditions.** *C. albicans* SC5314 (19) and SGY243 (27) were obtained from Squibb Pharmaceuticals. Strain S3153A (2) was obtained from D. R. Soll, and strain ATCC 35696 was obtained from P. Sundstrom. The strains were grown and maintained on YEPD medium (56) at 30°C.

In experiments comparing yeast and hyphal forms of the organism, the medium of Lee et al. (32) was used. The cells were grown to the stationary phase in the latter medium at 25°C and inoculated into fresh medium at a density of 5 x 10⁶ cells per ml. The cultures were incubated at 25 or 37°C to promote growth of the yeast or hyphal form, respectively. The cultures were harvested after 3 h of incubation.

**Southern and Northern (RNA) blot hybridizations.** Southern and Northern blots were prepared by standard procedures (52) with nylon membranes. DNA samples were separated on 1% agarose gels with either TAE buffer (52) or the buffer of Helling et al. (22). RNA samples were separated on 1% agarose gels with the formaldehyde buffer described by Lehrach et al. (33), and transcript sizes were determined relative to a 0.24- to 9.5 kb RNA ladder (GIBCO BRL). Electrophoretic separation of chromosomes by contour-clamped homogeneous electric field (CHEF) gel electrophoresis was conducted as described previously (36), except that 0.9% agarose gels were used and electrophoresed at 120 V with a 2-min switch for 20 h, a 5-min switch for 22.5 h, and a 20-min switch for 50 h at 80 V. Genomic DNA for Southern analysis was prepared by the method of Scherer and Stevens (54). RNA samples were prepared as described by Langford and Gallwitz (30). DNA fragments used as hybridization probes were purified by agarose gel electrophoresis and extracted with Gene Clean (Bio 101) prior to random-primer labelling with [α-³²P]GTP (16). A plasmid bearing the 27A repeat sequence (54) was kindly provided by S. Scherer, and the lambda Ca3 repetitive DNA (51) was generously provided by D. R. Soll. The *C. albicans* actin gene was provided by W. S. Rigsby (36a). Hybridizations were conducted at 42°C in 5x SSPE (1x SSPE = 0.18 M NaCl, 10 mM NaPO₄, and 1 mM EDTA [pH 7.7]-50% formamide-5x Denhardt’s solution-0.1% sodium dodecyl sulfate (SDS)-100 μg of denatured herring sperm DNA per ml-10⁻⁶ cpm of hybridization probe per ml. After overnight hybridization, the blots were washed twice in 0.2x SSC-0.1% SDS at 42°C for 10 min.

**Isolation of the alpha repeated element.** The alpha repeated element was fortuitously isolated from strain CA38-1A. This strain is a derivative of strain SGY243 containing an integrated copy of plasmid pCAR-1 (26). To examine the integration site, we recovered the integrated vector and adjacent genomic sequences as described by Stiles (61) following digestion of CA38-1A genomic DNA with *SalI*. The recovered plasmid, pCA381A-S2, contained approximately 1.7 kb of genomic DNA adjacent to the integration site. The genomic sequences contained in pCA381A-S2 were used as a hybridization probe to screen a lambda phage library to obtain a clone of the uninterrupted integration site. The library consisted of a partial Sau3A digest of genomic DNA from strain SC5314 ligated into the XbaI site of ΛGEM12 (Promega) in accordance with the manufacturer’s specifications. Hybridization screening of the library was conducted as described previously (5). A 1.3-kb EcoRI fragment containing the pCAR-1 integration site was subcloned from the insert of one of the hybridizing phage. This 1.3-kb EcoRI fragment was found to hybridize with multiple DNA fragments in Southern blots of EcoRI-digested genomic DNA. Various fragments of the 1.3-kb EcoRI clone were tested in Southern blot hybridizations to localize the repetitive sequences. These were localized to a 500-bp *EcoRV-NdeI* fragment. The *EcoRV-NdeI* fragment was cloned into the SpeI-EcoRV sites of plasmid pBSK+ (Stratagene). The 5’ overhanging ends of the SpeI and NdeI sites were made flush with Klenow polymerase (52). The resulting plasmid, pAlpha-1, contained the repetitive sequences flanked by SpeI and EcoRV restriction sites. The insert from pAlpha-1 was subsequently used as a hybridization probe to screen the ΛGEM12 genomic library to obtain molecular clones of the other genomic loci containing the repetitive element. One of these clones, λC3-3, contained a composite element designated Tca1.

**Nucleotide sequence analysis.** The nucleotide sequences of appropriate subclones were determined by dideoxy chain termination reactions (53) with Sequenase (U.S. Biochemical Corp.) or the ExoMeth procedure (59). Nucleotide sequence analysis was performed with the Wisconsin Genetics Computer Group sequence analysis software package, version 7.0 (10). Homology searches of the GenBank data base were conducted with the FASTA program of Pearson and Lipman (45).

The nucleotide sequences of the solo alpha element, the 5’ LTR of Tca1, and the 3’ LTR of Tca1 have been deposited in the GenBank data library under the accession numbers M94627, M94628, and M94629, respectively.

**RESULTS**

The alpha element is a dispersed repetitive sequence. We fortuitously isolated from *C. albicans* a 500-bp fragment of
EcoRI fragments with the designated genomic element.

Seven of the genomic DNA that contained a moderately repetitive sequence (see Materials and Methods). The repeated element was designated alpha. A restriction map of the alpha element and Southern blot hybridizations with the element as a hybridization probe are shown in Fig. 1. Southern blots of genomic DNA from strain SC5314 restricted with EcoRI showed eight bands of variable intensity when hybridized with the alpha element. The hybridizing fragments ranged in size from 1.3 kb to approximately 15 kb. The 1.3-kb fragment corresponded in length to the cloned genomic locus from which the alpha element was isolated.

The pattern of hybridization bands was strain specific (Fig. 1). Genomic DNA from strain SGY243 contained 10 EcoRI fragments that hybridized with the alpha element. Seven of the hybridization bands were identical in size to fragments detected in SC5314 DNA, while the remaining three were different in size. Similarly, EcoRI-digested genomic DNA from strain 3153A contained eight hybridizing fragments, several common to SC5314 and SGY243 genomic digests and several unique. The most distinctive pattern was obtained with strain ATCC 38696. DNA from this strain also contained eight hybridizing EcoRI fragments, only one of which was similar in length to fragments present in the other three strains.

To determine whether the alpha element was dispersed in the genome or localized to a specific region, we separated the chromosomes of strains SC5314, SGY243, and 3153A by CHEF gel electrophoresis and hybridized Southern blots of the gel with the alpha element (Fig. 2). Of the 10 chromosomal bands resolved from strain SC5314, 5 hybridized with the alpha element. Of the nine chromosomal bands resolved from strains SGY243 and 3153A, four bands and five bands, respectively, hybridized with the element. Thus, the alpha element appears to be dispersed in the genome.

**Direct repeats of alpha flanking a second repeated element.** To learn more about the structure and genomic organization of the alpha element, we isolated DNA clones of other genomic loci containing the alpha element. We did this by using the alpha element as a probe for plaque hybridization screening of a λGEM12 (Promega) library prepared from genomic DNA of strain SC5314. The genomic DNA insert of one of the phage isolates, λCJY-3, contained two EcoRI bands that hybridized with the alpha element (Fig. 3). These two bands were identical in length to two of the hybridization bands observed in Southern blots of genomic DNA, approximately 3.4 and 3.7 kb. A restriction map of the λCJY-3 insert spanning the two EcoRI restriction fragments is shown in Fig. 4. As seen in the restriction map, the two regions homologous to the alpha element were in the same orientation and separated by a region approximately 5.5 kb in length.

To determine whether the intervening region separating...
the alpha elements in \(\lambda\)CJY-3 contained unique sequences or was associated with other copies of the alpha element, we hybridized DNA fragments from the intervening region with Southern blots of EcoRI-digested genomic DNA (Fig. 5). Hybridization probes were prepared from regions either 5' or 3' of the EcoRI site located within the intervening region. The 1.8-kb \(\text{HindIII}-\text{EcoRI}\) fragment derived from the region 5' of the EcoRI site (Fig. 4) hybridized with two DNA fragments in EcoRI digests of SC5314 DNA. One band was 3.4 kb in length and corresponded in size to the 5' EcoRI fragment of \(\lambda\)CJY-3. The second hybridization band was 3.25 kb in length. An EcoRI fragment of identical size was detected with the alpha element as a hybridization probe. When the 0.8-kb \(NdeI\) internal fragment, located 3' of the EcoRI site in \(\lambda\)CJY-3 (Fig. 4), was used as a probe, two hybridization bands were detected again. One band, 3.7 kb in length, corresponded to the cloned genomic region in \(\lambda\)CJY-3. The second hybridization band, 4.8 kb in length, corresponded in size to a hybridization band detected with the alpha element. Thus, it appeared that a second copy of the alpha-associated sequence from \(\lambda\)CJY-3 was present in the genome of strain SC5314. A similar analysis of strain SGY243 demonstrated a hybridization pattern identical to that of strain SC5314 (Fig. 5). The pattern differed only in that the 3.25- and 4.8-kb bands were less intense than the 3.4- and 3.7-kb bands. These data suggested that strains SC5314 and SGY243 both contain at least two copies of the alpha-associated sequence and that these are similarly located in the genomes of both strains.

Analysis of strains 3153A and ATCC 38696 yielded different results. Southern blots of EcoRI-digested genomic DNA from 3153A exhibited a single band 3.25 kb in length when
hybridized with the 1.8-kb HindIII-EcoRI probe. This hybridization probe detected an identically sized band in digests of SC5314 and SGY243 DNA. Similarly, hybridization with the 0.8-kb NdeI probe revealed a single 4.8-kb fragment in EcoRI digests of 3153A, a hybridization band of identical size having been detected in SC5314 and SGY243. ATCC 38696 was unique in that a single 2.9-kb band was detected with the 1.8-kb HindIII-EcoRI probe and a single 4.0-kb band was detected with the 0.8-kb NdeI probe. While both of these bands were also detected with the alpha element as a hybridization probe, no fragments of equivalent size were detected in the other three strains. The data suggested that the alpha-associated sequence found in \( \lambda \)CY3-3 was located at a single locus in strain 3153A and that this locus was common to SC5314 and SGY243. The alpha-associated sequence in ATCC 38696, however, may be located at a different locus than in the other three strains. Alternatively, restriction site polymorphisms could be responsible for the unique sizes of the hybridization bands detected in ATCC 38696.

The HindIII-EcoRI and NdeI probes from \( \lambda \)CY3-3 hybridized with two EcoRI fragments in strains SC5314 and SGY243. These two hybridization bands may represent restriction site polymorphisms at a single homozygous locus or may represent sequences present at two different loci. To resolve this question, we hybridized a Southern blot of chromosomes separated by CHEF gel electrophoresis with the 0.8-kb NdeI fragment from \( \lambda \)CY3-3 (Fig. 6). In both SC5314 and SGY243 samples, two chromosomal bands of very different size hybridized with the probe. \( \lambda \)CY3-3 sequences flanking the region bounded by the alpha elements hybridized with the larger of the two chromosomal bands only (data not shown), indicating that these are not homologous chromosomes that differ in size. Therefore, the alpha-associated sequence appeared to be present at two different chromosomal loci.

Nucleotide sequence of the alpha elements. The arrangement of the alpha elements in \( \lambda \)CY3-3, direct repeats flanking a central region of 5 to 6 kb, is similar to the structure of retrotransposons and retroviruses (3, 65). The sequences within and around the LTRs of retrotransposons and retroviruses have characteristic features related to their mechanism of transposition (3, 65). If the sequences present in \( \lambda \)CY3-3 represent a retrotransposon-like element, then the conserved features of LTRs should be revealed in the nucleotide sequence of the alpha elements. Consequently, the nucleotide sequence of the alpha elements was determined and compared with that of the terminal repeats of other retrotransposons.

The two alpha elements contained in \( \lambda \)CY3-3 were 388 nucleotides in length and identical in sequence (Fig. 7). The solo alpha element initially isolated was 389 nucleotides in length because of the insertion of a G residue at position 132 of the sequence. This solo element also contained a T-to-A transversion at position 19. This alteration generated the EcoRV restriction site present in the solo element but absent from the alpha elements associated with the composite element. Each alpha element contained a 6-bp inverted repeat, 5'-TGTTTCG...CGAAACA-3', comprising the 5' and 3' borders. This inverted repeat is similar to those of S. cerevisiae sigma elements (TGGTTGAT) and delta elements (TGGTGGAA) and Drosophila copia elements (TGGT GAATA) (7). A polypurine tract, GAATCACGGAG, was located within the intervening sequence of \( \lambda \)CY3-3 immediately 5' of the 3' alpha element. This polypurine tract is postulated to serve as a plus-strand primer-binding site in retrovirus replication (65). Integration of retrotransposon-like elements results in a 4- to 6-bp duplication of the target site such that the integrated element is flanked by direct repeats of the target site (3). The 5' alpha element of \( \lambda \)CY3-3 was preceded by the 5-bp sequence 5'-TTGCT-3', which
was duplicated as a direct repeat at the 3' border of the 3' alpha element. In an analogous manner, the solo alpha element was flanked by direct repeats of the 5-bp sequence GATTA. These sequence features suggested that the alpha elements and the associated intervening sequence in λCY-3 comprise a composite element analogous to the retrotransposons of \textit{S. cerevisiae}.

**Regulation of Tac1 expression.** If the direct repeats of the alpha elements and the intervening sequence of λCY-3 comprised a retrotransposon-like element, then one would predict a transcript of at least 6 kb in length that would hybridize with the alpha elements as well as the intervening region. To test this possibility, we hybridized either the alpha elements or the intervening region from λCY-3 with Northern blots of total RNA. The 1.8-kb HindIII fragment from the internal region of the presumptive retrotransposon hybridized with a transcript approximately 6.9 kb in length (Fig. 8). The same hybridization band was detected when the Northern blots were hybridized with a DNA fragment containing only the alpha elements. The 6.9-kb transcript was evident in all four strains tested. These results indicated that the alpha elements and intervening sequence in λCY-3 are expressed as a composite transcriptional unit, as would be predicted for a retrotransposon-like element. Strand-specific probes demonstrated the composite element to be transcribed from left to right, as depicted in Fig. 4 (data not shown).

While the 6.9-kb transcript was the major hybridization band detected, additional hybridization bands of lesser intensity were also apparent (Fig. 8). Several of these hybridization bands were detected with both hybridization probes, but several hybridized with the alpha elements only. The pattern of alpha-specific transcripts was similar in strains SC5314, SGY243, and 3153A but differed slightly in strain ATCC 38696.

The expression of the 6.9-kb transcript was modulated in response to the growth conditions. The amount of the transcript was greatly increased in cells grown at 25°C relative to cells grown at 37°C (Fig. 8). Laser densitometric scanning of the autoradiographs indicated a 20- to 30-fold difference. This response was evident in all four strains tested, indicating a common mode of regulation. Temperature had no effect on the level of actin mRNA, indicating that there was not a general decline in transcription.

Under the growth conditions used in this experiment, strain SC5314 grew as yeast cells at 25°C but grew as hyphae at 37°C. Strain SGY243, however, did not exhibit the dimorphic transition under these growth conditions and grew in the yeast form only. Consequently, the expression of the retrotransposon-like element appeared to be independent of morphology. A number of other growth media were tested, with similar results (data not shown). Several of the alpha-specific transcripts were also modulated in amount in response to temperature (Fig. 8).

The alpha element is a novel repeat from \textit{C. albicans}. A number of dispersed, moderately repetitive sequences have
been isolated from *C. albicans*. These include the repetitive element Ca3 (51) and the related 27A repeat (54), the telomere-associated Ca7 repeat (51), CARE-1, described by Lasker et al. (31), and the *MspI* repeat described by Cutler et al. (9). Tca1 does not appear to be related to any of these sequences. The alpha element does not cross-hybridize with either Ca3 or 27A (data not shown). The sequence of the element contains no homology with the reported sequence of CARE-1, and the restriction map is distinct from that of the *MspI* repeat. Since the alpha element did not hybridize with all of the chromosomes (Fig. 2), it cannot be analogous to the Ca7 repeat. The copy number of the composite Tca1 element also clearly distinguishes it from these other repetitive sequences. Comparison of the nucleotide sequence of the alpha element with the GenBank data base also failed to reveal any similar sequences.

**DISCUSSION**

Our investigation of a dispersed repetitive sequence, which we called alpha, led to the isolation of a composite element containing two copies of the alpha element. We have chosen to designate the composite element Tca1 (transposon *Candida albicans*). Several features of this element suggest that it is a mobile element analogous to the retrotransposons of other organisms. The gross structure of Tca1, LTRs flanking a region of approximately 5.5 kb, is characteristic of retrotransposons and retroviruses (3, 65). The LTRs of Tca1 were identical direct repeats 388 nucleotides in length. The sequence of these alpha elements began with the nucleotides GT and ended with the nucleotides CA, a feature conserved in the LTRs of retrotransposons and retroviruses (66). As in other LTRs (66), these delimiting nucleotides were part of an inverted repeat. This inverted repeat was 6 nucleotides in length in the alpha elements. The 3′ alpha element was preceded by a polypurine tract within the internal domain of the composite element. This polypurine tract is thought to provide the plus-strand primer-binding site for reverse transcription of retrotransposable elements (65). In addition to these features, both the alpha elements and the internal domain of Tca1 hybridized with the same 6.9-kb transcript, as expected of a retrotransposon-like element. Transcription of retrovirus-like elements initiates within the 5′ LTR and terminates within the 3′ LTR, generating a terminally redundant mRNA (3, 65). Finally, sequence analysis demonstrated that Tca1 was flanked by a 5-bp direct repeat. Such direct repeats are characteristic of transposable elements and result from a duplication of the integration target site (3, 65).

The one conserved feature of retrotransposons and retroviruses that was not clearly evident in Tca1 was the minus-strand primer-binding site. This region of 8 to 18 nucleotides is located immediately adjacent to the left LTR in retrotransposons Ty1 and Ty2 of *S. cerevisiae* (3) or 2 nucleotides 3′ of the left LTR in Ty3 (3, 7) and retroviruses (66). Complementarity between the minus-strand primer-binding site and the 3′ end of a specific tRNA allows the tRNA to act as a primer for reverse transcription. The sequence adjacent to the left LTR of Tca1 did not contain a 5′-TGG-3′ sequence complementary to the conserved CCA sequence at the 3′ end of tRNA molecules. This result suggests that the priming of reverse transcription of Tca1, if it occurs, may be atypical and perhaps similar to that of the copia retrotransposon of *Drosophila melanogaster*. The primer used by copia elements is a cleavage product of initiator methionine tRNA containing only the first 39 nucleotides (28). Interestingly, the 9 nucleotides immediately adjacent to the left LTR of Tca1 perfectly complement nucleotides 31 to 39 of Arg-tRNA-3 of *S. cerevisiae* (25). This fact may indicate that a cleavage product of tRNA*aa* serves as the primer of Tca1, since components of the translational apparatus are highly homologous between *S. cerevisiae* and *C. albicans* (42, 63).

The alpha element initially isolated was not associated with a Tca1 element. With the exception of two base pair changes, this solo alpha element was identical in sequence to the alpha element associated with Tca1. Like Tca1, the solo alpha element was flanked by a 5-bp direct repeat. However, these flanking nucleotides were different from those flanking the cloned Tca1 element. This solo alpha element appears to be analogous to the solo LTRs of *S. cerevisiae* Ty elements. These solo LTRs appear to be derived via intrachromosomal recombination between the direct repeats of the retrotransposon (46). This recombination event results in excision of the intervening sequence (46). As a consequence, the remaining solo LTR is flanked by the 5-bp target site duplication that occurs during integration of the retrotransposon (15). Since intrachromosomal recombination between direct repeats, with excision of the intervening sequence, has been demonstrated for *C. albicans* (20), the solo alpha element could have been generated from a Tca1 element by this type of recombinational event.

The exact copy number of Tca1 and solo alpha elements is uncertain. Since *C. albicans* is diploid, individual hybridization bands observed on Southern blots cannot be interpreted as representing individual chromosomal loci. A pair of hybridization bands may represent a single locus heterozygous for a restriction site polymorphism (27). Even when a hybridization band corresponds to an individual locus, that locus may be heterozygous or homozygous for the element. Tca1 is present at two loci in strains SC5314 and SGY243, as indicated by Southern blot analysis of electrophoretically separated chromosomes. Thus, there are at least two copies of Tca1 in these strains, but there could be as many as four if both loci are homozygous. The other strains examined contain either one or two copies of Tca1. The low copy number of the Tca1 element may reflect a low rate of transposition, removal of transposed copies by gene conversion, or a selective disadvantage of most integration events.

The copy number of the solo alpha element appears to be slightly higher than the copy number of the Tca1 element. Strain SC5314 contains a minimum of four copies of the solo alpha element, since Southern blots exhibited four hybridization bands not associated with the Tca1 element. There may be as many as eight copies if all four hybridization bands represent individual, homozygous loci. A similar analysis indicated that the other strains used in this study contain a minimum of 6 and a maximum of 12 copies of the solo alpha element. These copy numbers are comparable to those of the yeast Ty3 element (7) but much lower than those of the Ty1 element. Laboratory strains of *S. cerevisiae* contain approximately 30 copies of Ty1 and as many as 100 copies of solo delta sequences (3, 47). However, natural yeast isolates which, like *C. albicans*, are normally diploid, contain only a few copies of Ty1 (12).

The expression of Tca1 was strongly regulated by temperature, a parameter that may have particular significance in relation to strain virulence. The virulence determinants of a number of pathogenic bacteria are known to be regulated by temperature, and these include genes that are either down-regulated or up-regulated at the host temperature (38). The virulence of *C. albicans* is also affected by temperature. *C. albicans* cells grown at 25°C are more virulent than those
grown at 37°C (1). Dissection of the regulatory pathway controlling Tca1 expression could lead to the identification of virulence genes that are subject to the same temperature-dependent regulation. Of equal relevance to virulence may be the active transposition of the Tca1 element. Just as yeast Ty elements can regulate adjacent gene transcription (3, 47), the Tca1 element may place adjacent genes under temperature-dependent control. Since Tca1 exhibits higher expression at temperatures below that of the human host, Tca1 control could allow for increased expression of genes that improve survivability outside the host or increased expression of genes required for the establishment of a host infection, analogous to the expression of the invasion gene of Yersinia pseudotuberculosis (23). Alternatively, Tca1 could allow for the down-regulation of genes that trigger host defenses, analogous to the effects of the IS476 mobile element of Xanthomomas campestris (24). In this context, it should be noted that a number of temperature-regulated, alpha-specific transcripts were observed on Northern blots. This result suggests that the alpha element may act as a temperature-dependent activator of adjacent gene transcription.

A retrotransposable element such as Tca1 not only may have a role in the evolution of genomic diversity in C. albicans but also may provide a means of categorizing and defining that diversity. The solo delta sequences of S. cerevisiae undergo mutational alterations but are rarely, if ever, excised (47). Assuming that the solo alpha element of C. albicans behaves in a similar manner, it may provide fixed genetic markers that delineate C. albicans strain lineages. In this study, strains SC5314 and SGY243 appeared to be closely related. The eight alpha-hybridizing bands detected in strain SC5314 were also present in strain SGY243. SGY243 contained only two additional hybridization bands not detected in SC5314. Both strains also contained Tca1 elements at the same two loci. Strain 3153A also appeared to be related to strains SC5314 and SGY243, but less closely. Only six of the eight alpha-hybridizing bands in 3153A were shared with the latter two strains, and 3153A contained only a single copy of Tca1. Strain ATCC 38696 was the least related of the four strains and contained only a single alpha-hybridizing band in common with the other three strains. Other genetic characteristics of these strains appear to substantiate these relationships (17), suggesting that alpha element patterns may provide a means of defining genetically related C. albicans isolates. The significance of Tca1 in the biology of C. albicans is under further investigation.

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