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The structure and retrotransposition mechanism of LTR-retrotransposons in the asexual yeast *Candida albicans*

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Retrotransposons constitute a major part of the genome in a number of eukaryotes. Long-terminal repeat (LTR) retrotransposons are one type of the retrotransposons. *Candida albicans* have 34 distinct LTR-retrotransposon families. They respectively belong to the *Ty1/copia* and *Ty3/gypsy* groups which have been extensively studied in the model yeast *Saccharomyces cerevisiae*. LTR-retrotransposons carry two LTRs flanking a long internal protein-coding domain, open reading frames. LTR-retrotransposons use RNA as intermediate to synthesize double-stranded DNA copies. In this article, we describe the structure feature, retrotransposition mechanism and the influence on organism diversity of LTR retrotransposons in *C. albicans*. We also discuss the relationship between pathogenicity and LTR retrotransposons in *C. albicans*.

Introduction

As an opportunistic fungal pathogen of humans, *Candida albicans* usually cause fungal infections such as thrush, vaginitis, and life-threatening bloodborne candidiasis.[1] Of the 16 Mb haploid genome of *C. albicans*, 14.9 Mb has been assembled by Stanford University (Stanford DNA Sequencing and Technology Center), which facilitates understanding of the genetic structure, pathogenicity, adaptability, and evolution in *C. albicans*. Retrotransposons are mobile genetic elements capable of independent transposition through RNA intermediates.[1] Retrotransposons are widespread transposable elements in eukaryotes, and constitute a major part of eukaryotic genomes in many cases.[2-7] For instance, retrotransposons constitute 42% of human genomes[8] and 75% of maize genomes.[9]

Two primary subclasses of retrotransposons are present in eukaryotic cells: long-terminal repeat (LTR) retrotransposons and non LTR retrotransposons (LINEs, long interspersed nuclear elements, and SINEs, short interspersed nuclear elements).[10]

LTR retrotransposons in the yeast *Saccharomyces cerevisiae* have been extensively studied. According to the sequence similarity of reverse transcriptases (RTs)[11,12] and the subunits of *pol* genes, there are two different types of LTR retrotransposons.[1] One is the *Ty1*/*copia* type, where the subunits are protease (PR), integrase (IN), reverse transcriptase (RT), and RNase H (RH) in order; the other is the *Ty3*/*gypsy* type, where the subunits are PR, RT, RH, and IN (**Fig. 1**).[13] In *S. cerevisiae*, six classes of retrotransposons, *Ty1–5* and *Ty3p*, belong to 2 different types, respectively: *Ty1*/*copia* components (*Ty1*, *Ty2*, *Ty4*, and *Ty5*), and *Ty3*/*gypsy* components (*Ty3* and *Ty3p*). In these retrotransposons families, *Ty1*, *Ty2*, *Ty3*, and *Ty4* have transposition activity in *S. cerevisiae* as known.[14] This review summarizes the structure, mechanism, and influence on organism diversity of LTR-retrotransposons found in *C. albicans*.

LTR-Retrotransposon Families in *C. albicans*

Compared with the 6 families present in *S. cerevisiae*, 34 distinct LTR-retrotransposon families pertaining to the *Ty1*/*copia* and *Ty3*/*gypsy* groups have been found in *C. albicans*.[15] They are named by the Greek alphabet letters (α, β,… *iota*.), archaic Greek letters (*sampi*, *san*, etc.), and phonetically similar names of New Zealand birds (*moa*, *tara*, *weka*, etc.).[15] According to the internal regions, the first 16 LTRs have been defined as *Tca1–Tca16* (**Table 1**).

By comparing the LTR retrotransposons families in *C. albicans* and *S. cerevisiae*, we can find a great difference.[15] The different number of families illustrates that the two species experience disparity in retrotransposons element evolution.

LTR retrotransposons contain two long-terminal repeats (LTRs), as shown in **Figure 1**, at their ends, typically 250–600 bp in length, flanking a 5–7 kb long internal protein-coding domain.[16] Between the two LTRs are two open reading frames (ORFs): *gag* and *pol*. The *gag* ORF encodes the structural proteins that make up a virus like particle (VLP).[17] The *pol* ORF encodes the enzymes required for reverse transcription and integration. The enzymes are PR, IN, RT, and RH.

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Table 1. Properties of *C. albicans* retrotransposon LTR families

LTR	Length (bp)	TSD (bp)	Associated internal region	Copy number[a]	Accession number	Gag/pol ORFs[b]
alpha	388	5	Tca1	10 (5–10)	M94628	No
beta	395	5	Tca8	10 (6–8)	Y08494	Partical
gamma	280	5	Tca2	9 (5–10)	Y08494	Intact
kappa	280	5	Tca6	20 (10–15)	AF069450	Frag.s
zeta	508	5	Tca7	19 (10–15)	AF074943	Partial
san	381	5	Tca4	5 (1–4)	AF074943	Intact
omega	685	5	Tca5	3 (0–5)	AF093417	Intact
nu	277	4	Tca3	11	AF119344	Partial
psi	470	5	Tca9	30	AF119344	No
chi	192	5	Tca10	11	AF118059	No
eta	470	5	Tca11	13	AF118059	N.D.
whio	348	5	Tca12	8	AF180289	N.D.
moa	507	5	Tca13	8	AF180291	No
lambda	512	5	Tca14	4	AF180284	N.D.
kahu	531	5	Tca15	17	AF192278	Partial
huia	127	5	Tca16	6	AF180285	Frag.s
omicron	268	4	-	9	-	-
rho	275	4	-	13	-	-
pi	280	4/5	-	17	-	-
lota	251	4	-	13	-	-
sampia	324	5	-	9	-	-
theta	366	5	-	7	-	-
upsilon	264	5	-	6	-	-
koppa	208	5	-	10	-	-
epsilon	480	5	-	9	-	-
phi	194	5	-	14	-	-
episemon	518	5	-	4	-	-
mu	780	5	-	4	-	-
xi	387	5	-	5	-	-
weka	165	5/7	-	9	-	-
tui	199	5	-	19	-	-
titi	336	5	-	3	-	-
tara	285	5	-	9	-	-
toroa	282	5	-	11	-	-

[a]Numbers in parentheses indicate the copy number in a variety of strains as estimated by Southern blotting. [b]Intact, long ORFs present containing all of the motifs characteristic of full-length, functional retrotransposons; Partial, long ORFs are present but no full-length ones discovered yet; Frag.s, no long ORFs, but short regions of homology to other retroelement ORFs are present; No, no long ORFs present nor any homology to other retroelements in databases.

The LTRs of retrotransposons have the transcription termination signals and promoter, and are divided into three functional areas: U3, R, and U5.[17] U3 contains an enhancer and promoter region at the 3′ end of transcript; R contains both the start and termination sites for transcription;[18] U5 only exists in the transcript of 5′ terminal. The process of transcription is from the left LTR U3/R boundary to the right LTR R/U5 boundary to produce a RNA molecule with both ends of R region (**Fig. 2**).

Thirty-four LTR retrotransposon families in *C. albicans* share the following characteristics: (1) the length of the retrotransposon LTRs ranges from 127 to 780 bp with a mean length of 359 bp; (2) most elements have the terminal dinucleotides 5′-TG…CA-3′ and these dinucleotides tend to form part of larger terminal inverted repeats; (3) the total cope number of LTRs is 355; (4) both sides of more than half of the full-length LTR are short direct repeats representing target-site duplications (TSDs),

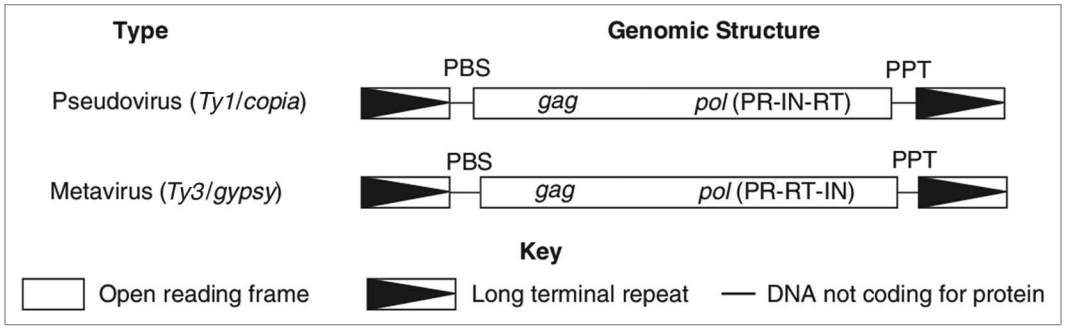

Figure 1. The genomic organization of different types of LTR-retrotransposon families present in *C. albicans*. RT, reverse transcriptase DNA polymerase domain; RH, reverse transcriptase RNase H domain; PR, proteinase; IN, integrase.

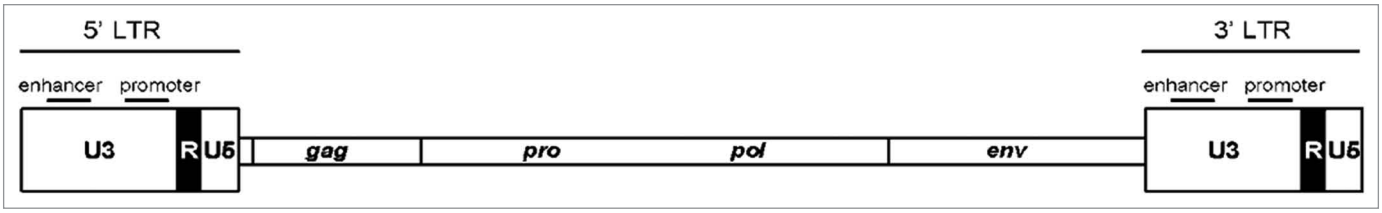

Figure 2. LTRs can be subdivided into three regions: U3, R, and U5. U3 contains the enhancer and promoter sequences that drive viral transcription. R domain encodes the 5′ capping sequences (5′ cap) and the polyA (pA) signal.

most of which are 5 bp in length showing a tendency toward a purine in the first position and a pyrimidine in the last (data not shown);[15] and (5) these retrotransposon families in *C. albicans* generally vary widely in coding capacity as *Ty* families in *S. cerevisiae* and have a low copy number. Most families have the similar copy number to *Ty5* (7 copies) LTR in *S. cerevisiae*, but considerably lower than that of *Ty1/Ty2* (251 copies), *Ty3* (41 copies), and *Ty4* (32 copies).[15] Most LTR families in *C. albicans* only contain solo LTRs or LTR remnants in which the LTR-retrotransposon sequence has been retained and the intervening sequence has been lost. So far, just 3 intact retrotransposons have been founded. They are (1) *Tca2*, an active retrotransposon element which has a stop codon between two ORFs,[17] (2) *Tca5*, a *Ty5* like retrotransposon,[19] and (3) *Tca4*, which belongs to *Ty1/copia* group and resembles to *Tca2*.[15] Several families of highly degenerate elements appear to be still capable of transposition, presumably via transactivation. Full-length retrotransposons are usually lost when two LTRs recombine with each other. This results in isolated solo LTRs at the original sites. A survey shows that 85% of *Ty* insertions in *S. cerevisiae* are solo LTRs or LTR fragments.[15] In *C. albicans*, for retrotransposon element, β, there are some 395 bp solo LTRs.[20] Both full-length retrotransposons and solo LTRs have short (4 or 5 bp) direct repeats on either end.[15] Most LTR retrotransposons generate 5 bp short direct repeats representing TSDs, as the *Ty* elements in *S. cerevisiae*.[15] For instance, 36% of the total *S. cerevisiae Ty1–4* elements are flanked by TSDs.[21] Except for *Ty3/gypsy* elements (*Ty3*) that are flanked by 4 bp TSDs,[22] *Ty5* elements have no TSDs. The condition of *Ty5* suggests frequent recombination between these elements at the telomeres.[23] Recombination or mutation may have resulted in exchange of target site sequences between elements. A similar preference is apparent for the elements in *C. albicans*: 14 full-length LTRs are flanked by 4 bp direct repeats, like element *pi*,[10] *weka* has a confirmed 7 bp TSD. With analysis of the 5 bp TSDs in *C. albicans* and *S. cerevisiae*, base bias can be found: purine highly is enriched in the first site (55%); and pyrimidine is enriched in the last site (53%) and there is a strong bias for A and T: in the internal position 2 (72%), position 3 (76%), and position 4 (78%) (data not shown).[15]

LTR retrotransposons have a potential tRNA primer-binding site. At the downstream from the left of 5′ LTR is a short polypurine sequence (8–49 nt), termed as the primer-binding sites (PBSs) (**Table 1**), which is a 10–20 nucleotide sequence that can base-pair with cytoplasmic tRNA molecule partly.[15] Fourteen different families of LTRs in *C. albicans* have connection with PBSs.

All PBSs share a common feature, the LTR terminus starting from 5′-TGG-3′ is base-pair with CCA sequence at the 3′ end of all tRNAs.[15] Goodwin et al.[15] had found the 3′ end of tRNA could be used as a primer to determine whether two LTRs were two independent insertions or a single retrotransposon. Furthermore, if the two LTRs belong to a family, they repeat in a direct orientation on a contig.[15] According to the above mentioned common features, 14 families of LTRs in *C. albicans* have been found sharing the relative PBSs. There are 7 different classes of these PBSs (**Fig. 3**): some have an extensive region which is homologous to an internal region of $tRNA^{Arg(UCU)}$ (e.g., *gamma*), some have a short region which is homologous to the same tRNA (e.g., *whio*), some are homologous to a $tRNA^{iMet}$ internal region (e.g., *zeta*), and some are homologous to the 3′ ends of various tRNAs such as, Ala, Gln, Ile, and iMet tRNAs.[15] In some instances, the PBS is found to be located downstream of the LTR just 2–10 bp.

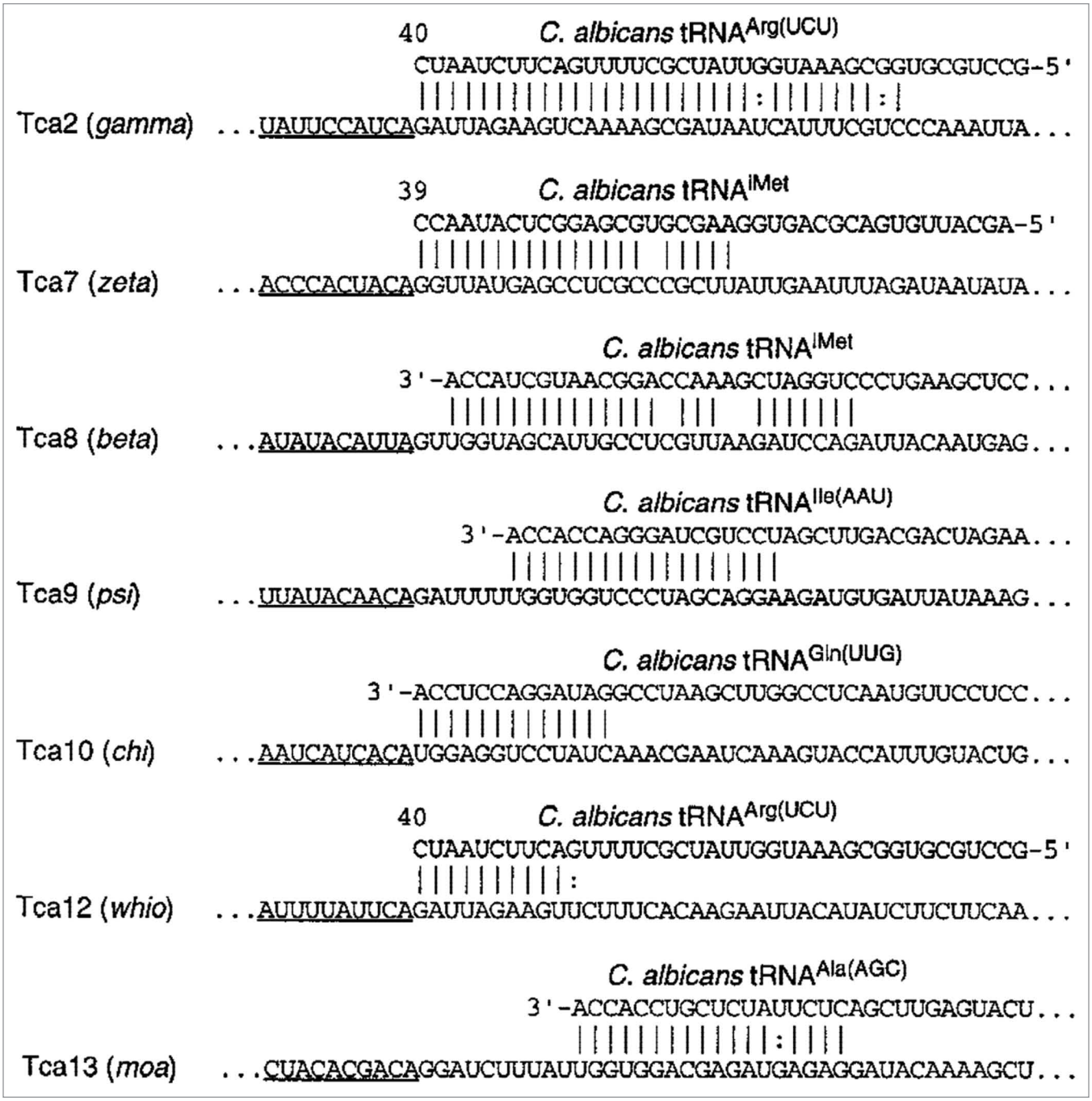

Figure 3. The diversity of PBSs in *C. albicans* retrotransposons. The names of the retrotransposons and the associated LTRs are shown on the left. LTR sequences are underlined. The GenBank accession numbers of the tRNAs are as follows: $tRNA^{Arg}$, AF041470; $tRNA^{iMet}$, AF069449; $tRNA^{Ile}$, Y08492; $tRNA^{Gln}$, AF180282; and $tRNA^{Ala}$, Y08493.

A short gap between the LTR and the PBS is a common feature of *gypsy*-class retrotransposons.[24]

Mechanisms of Retrotransposition

A general phenomenon that resides in a number of eukaryotes is that adverse living conditions can activate retrotransposons. They can move from place to place in a genome by reverse transcription of a RNA transposition intermediate to enable the organism to adapt to the environment. It has been demonstrated that the retrotransposons generate new copies by reverse transcription of their RNA transcripts.[3]

Similar to retroviruses in function, the LTRs of retrotransposons play a border role in complex reverse transcription procedure.[25] The two DNA strands are synthesized from opposite directions: tRNA binds to the site near the 3′ end of left LTR when synthesizing the first strand, while the polypurine track (PPT), a

short purine-rich sequence[17] immediately upstream of the right LTR, as a primer binds to the upstream of the right LTR when synthesizing the second strand.

Retrotransposons must synthesize mRNA at first, which can be translated into proteins related to replication, also acts as a replication template. At each end of the template, mRNA has a short repeat sequence (R, green) and U region (**Fig. 4**).[26] Primer tRNA can base-pair with PBS sequence. When tRNA anneals to PBS, minus strand DNA starts synthesizing under the catalysis of reverse transcriptase. DNA synthesized can reach the U5 region (red) at the 5′ end of the template and continue as the last few bases. The newly synthesized DNA can base-pair with the repeat sequence at the 3′ end of the genomic RNA.[26] Then, new strand DNA base-pair with 3′ end of the RNA template starting with U3 sequence, and continues until R and U5 at the 5′ end of RNA template. After that the RNase H degrades the RNA template leaving fragments at the poly-purine tract to prime second strand DNA synthesis. Using the new strand DNA as a template reverse transcriptase synthesizes another strand DNA until U5 and R. After that, the DNA fragment transfers to another end of new template. Synthesis then proceeds in both directions to give double-stranded DNA and LTR, making up of U5, R and U3, at each end.[26] Integrase inserts this into chromosomal DNA, and transcription initiating in one LTR and terminating in the other generates genomic RNA with terminal repeats.[26]

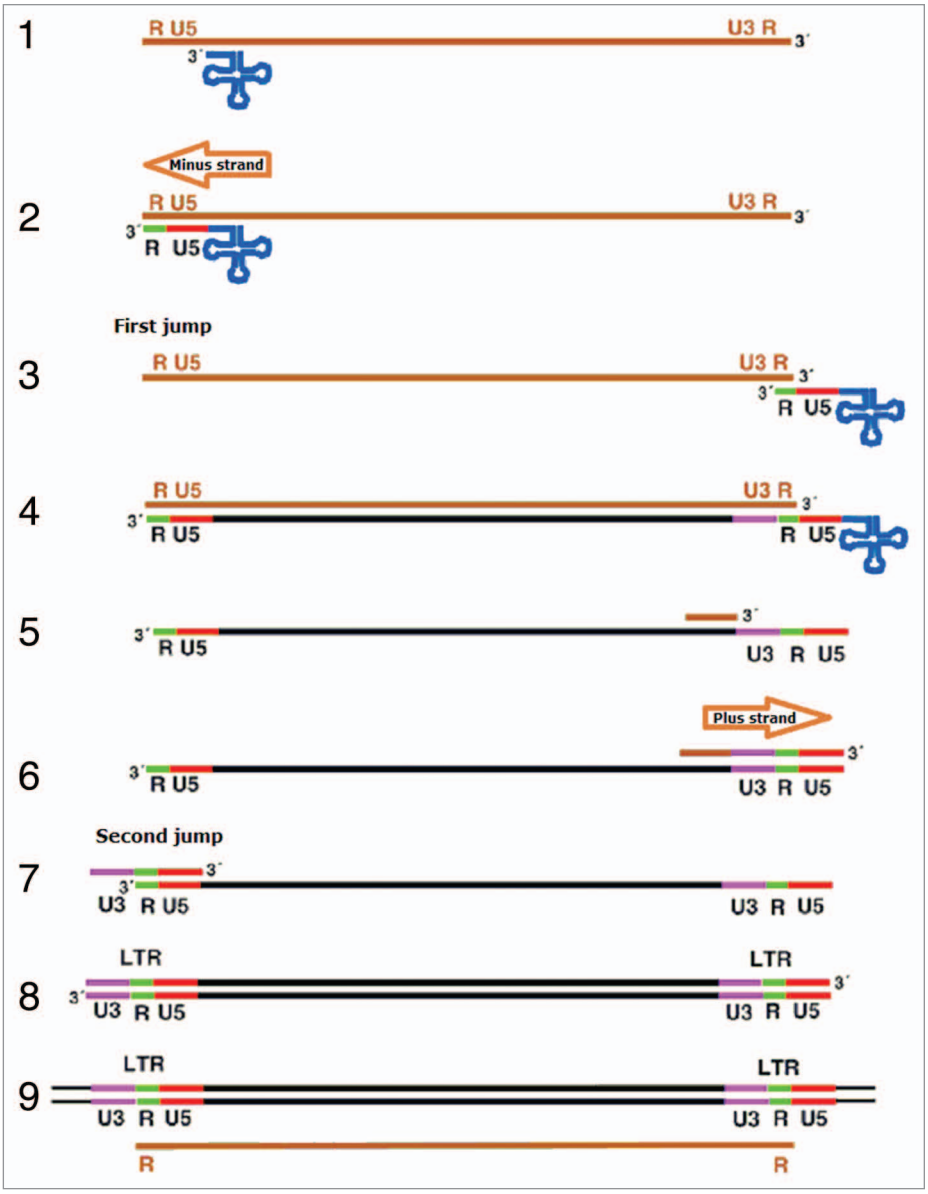

Figure 4. Mechanisms of retrotranspositions. The RNA transposition intermediate (brown) of an LTR retrotransposon provide plus strand RNA. The RNA has a tRNA (blue) base-paired sequence, the PBS, near its 5′ end (1). Primer tRNA anneals to binding site on RNA (2). The first sequences to be copied are the unique sequence at the 5′ end of the RNA (U5, red) and a short repeat sequence (R, green) present at both ends of the RNA. In this step, single-stranded DNA R region pairs with 3′ terminus in the first jump (3). Reverse transcriptase starts synthesis minus strand DNA (4), starting with U3 (purple) adjacent to R, and continuing until U5 and R are copied a second time. tRNA primer is removed. The RNA template is degraded by RNase H leaving a fragment at the poly-purine tract to prime second strand DNA synthesis (5). In the second jump, reverse transcriptase transfers to the other end of minus strand (7). Synthesis then proceeds (8). Integrase inserts this into chromosomal DNA, and transcription initiating in one LTR and terminating in the other generates genomic RNA with terminal repeats (9).

Specific Description

Of the 34 distinct LTR-retrotransposon families in *C. albicans*, 16 families still retain some internal sequences which have very different coding capacity (*Tca1*–*Tca16*). *Tca2*, *Tca4*, and *Tca5* are intact and possess all the characteristic features of functional retrotransposons. *Tca3* and *Tca8* have no full-length elements, but still retain long and continuous ORFs like other retroelements; *Tca6* still contains ORF fragments in the internal region; *Tca9* and *Tca13* have 5 bp direct repeats at each end, intact PBSs and PPTs and identical LTRs; *Tca10* is a compound element, whose LTR shares 99.5% identity and is flanked by a 5 bp direct repeat.[15] Some extensive ORFs, the PBS and PPT regions make up the ~2 kb long internal region.

The other 18 LTR families do not have the sequences similar to retrotransposons internal regions. These retrotransposon families may remain solo LTRs and LTR fragments, resulting in some of these LTRs escaping from detection.

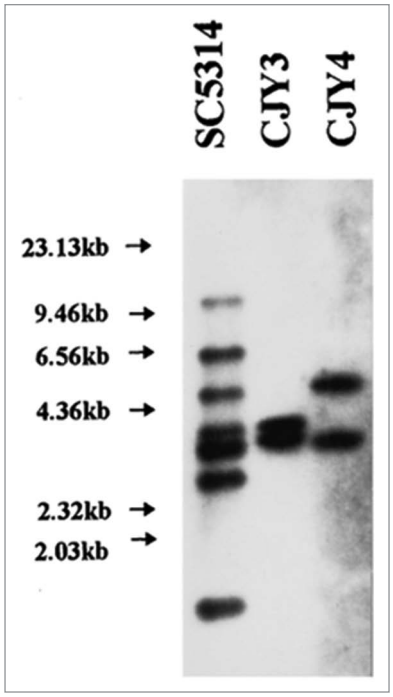

Figure 5. Southern blot analysis of DNA from lambda clones CJY-3 and CJY-4. Genomic DNA from strain SC5314 or purified DNA from lambda clones CJY-3 and CJY-4 was digested with EcoRI and hybridized with the α-element probe.

Many researchers have studied a few LTR retrotransposons in *C. albicans*. Now we summarize the characteristics of *Tca1*, *Tca2*, *Tca3*, *Tca4*, and *Tca5*.

Tca1, an Inactive Element without ORF

Tca1, as the first *C. albicans* retroelement, is of 5614 bp and has 388 bp LTRs.[15] No significant ORF sequence[27] presents in the internal region, suggesting that *Tca1* is a degenerate and inactive element. It has been demonstrated that *Tca1* has two loci in the strain SC5314. One is referred to as *Tca1–1*, and the other is designated as *Tca1–2*. The *Tca1* elements of the two loci have more than 99% similarity in sequence and almost the same structure.[27] *Tca1–1* was isolated from a lambda phage genomic library clone called CJY-3.[28] This clone contains 3.25 kb and 4.8 kb long fragments (**Fig. 5**). *Tca1–1* lacks any retrotransposon-like ORF. This differentiates *Tca1–1* from *Tca1–2*. *Tca1–2* was found in an approximately 6 kb clone, called CJY-4. It is highly similar to the insert of CJY-3, besides minor restriction site polymorphisms (**Fig. 6**).

Nucleotide sequence analysis suggests that the insert of CJY-4 is flanked by identical direct repeat sequences, which was 99% identical to the LTRs of *Tca1–1*. Each LTR of *Tca1–2* is flanked by 6 bp inverted repeat sequence (TGTTCG) like LTRs of other retrotransposons.[28] The sequence in the front of 5′ LTR and behind of 3′ LTR of *Tca1–2* is ATTGC. This suggests that the sequence is a copy of integration target site.[29] The sequence is different from the one of *Tca1–1*, TTGGT.

Sequence analysis[30] of entire *Tca1–2* has not found any extended ORFs or potential splice sites.[31] This suggests that *Tca1–2* has been degenerated highly. Compared with about 1400 bp of insertion region of *Tca1–1*, *Tca1–2* is just differing in less than 1% (data not shown).

Previous work[28] indicated that the transcription of *Tca1–1* is affected by growth temperature. Northern blot analysis suggested that the transcription of *Tca1* is also influenced by temperature in strains lacking *Tca1–1* or *Tca1–2*.[27] Thus, *Tca1* elements are temperature-regulated retrotransopsons. The expression level of *Tca1* at 25 °C is higher than that at 37 °C.

It has been reported that the virulence determinants of a lot of pathogenic bacteria (*Shigella app*, *Yersinia app*, *Vibrio cholerae*, *Bordetella pertussis*, and *Staphylococcus aureus*) were regulated by temperature.[32] Antley et al.[33] found *C. albicans* cells grown at 25 °C are more virulent than those grown at 37 °C. As *Ty* elements can regulate adjacent gene transcription[34] the *Tca1* may control adjacent genes temperature-dependently.[28] The expression of *Tca1* could be similar to the invasion gene in *Yersinia pseudotuberculosis*.[35]

Southern blot analysis suggests that deletion of *Tca1* elements does not result in obvious growth defects and the recombination between LTRs occurs readily.[27] Furthermore, silencing a single element would lead to complete loss at that locus, suggesting that the *Tca1* elements at both loci are hemizygous.[27] It is known that elements may be abundant in natural populations if they provide some advantage to the microorganism. However, there is no clear reason to maintain these degenerate elements. Neither copy of *Tca1* choose silent chromatin as preferential integration region as the *Ty5* retrotransposon in *S. cerevisiae*.[36-39] Although nearly 40% of the strains lack *Tca1* elements, solo LTRs in the genome suggest that they have existed in these strains for a time, and moreover, it is not essential to maintain *Tca1* in natural populations.[28]

Tca2 is an Abundant, Extrachromosomal DNA Molecule with Intact ORF

Tca2 is widespread in *C. albicans* and was first identified in the strain hOG1042, known as pCal. The pCal was a distinct band when the uncut *C. albicans* DNA was examined on an agarose gel, so it is an extrachromosomal retrotransposon.[17] *Tca2* belongs to *Ty1*/*copia*-type retrotransposon, and was originally identified as a linear double-stranded DNA molecule. It is the most abundant DNA copy (about 50 copies per cell).[16] *Tca2* is 6426 bp long and has 280 bp LTRs flanked by a six-nucleotide imperfect inverted repeat, TGTTGG…CCATCA. The internal domain usually has two long *gag* and *pol*-like ORFs.[16] ORF1 and ORF2 are separated by a stop-codon (UGA).[29] The structure feature is similar to mammalian retroviruses[40] but is unique in LTR retrotransposons.[13] However, the *pol* gene can translate into protein. Forbes and coworkers[41] demonstrated that the LTR promoter

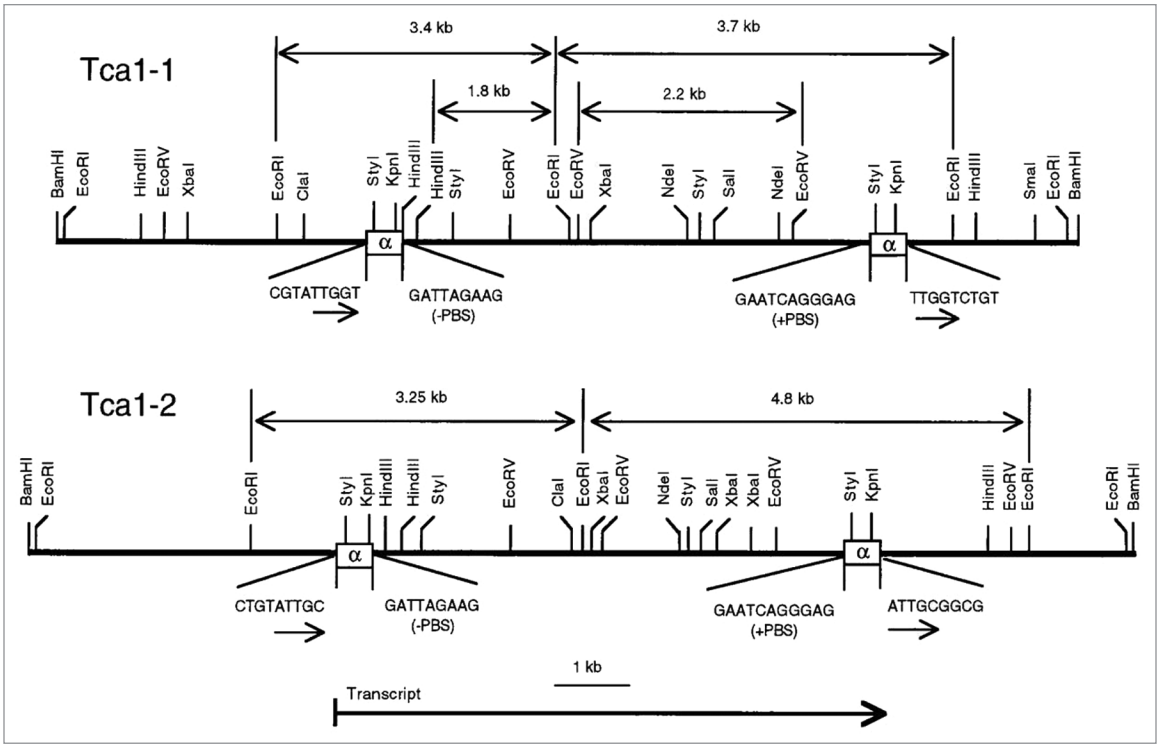

Figure 6. Restriction site maps of the genomic inserts from lambda clones CJY-3 and CJY-4 containing *Tca1–1* and *Tca1–2*, respectively. The locations of elements (LTRs) are indicated by the boxed regions. Also indicated are the plus- and minus-strand primer binding sites (1PBS and 2PBS), the 5 bp direct repeats flanking the elements, the EcoRI fragments associated with each locus, and the 1.8 kb HindIII-EcoRI and 2.2kb EcoRV fragments that are used together as a hybridization probe of the internal region. The direction of transcription of *Tca1* is from left to right.

is heat-shock induced. Like *pol* proteins, the expression of *Tca2 pol* protein is regulated by LTR promoter. There is a novel stop codon bypass mechanism to made *gag-pol* RNA translate *pol* proteins.[13] The mechanism is read-through suppression of the UAG codon.[17] In *Tca2*, the sequences between the *gag* and *pol* ORFs are an 8 bp purine-rich sequence, AAAACAGG, and a potential pseudoknot.[42] They are tightly followed the UGA stop codon, which is essential to suppression.

Tca2 has retrotranspositional activity, and is strongly dependent on the growth temperature.[16] There is substantially more extrachromosomal DNA at 37 °C than that at 27 °C. In addition, northern blot analysis on the transcriptional activity of *Tca2* showed that the most prominent band is full-length transcripts. Interestingly, the number of full-length transcripts at 37 °C is more than that at 27 °C, suggesting that *Tca2* has transposition activity and is in favor of higher temperature (37 °C). There is also a correlation between the level of *Tca2* RNA and extrachromosomal DNA, suggesting that the synthesis of extrachromosomal DNA needs *Tca2* RNA as intermediate. The level of *Tca2* RNA is relative to the situation of *Tca2* elements. For instance,[16] the levels of *Tca2* RNA are different at various sites in genome. In addition, the levels of RNA are disparate in different strains.[16] The level of transcription is probably affected by strains, sequences and genomic context. Thus, we speculate that there may be close relationship between the *Tca2* expression and virulence in the process of *C. albicans* evolution.

Tca3 has a Partial Coding Region

Tca3 is a *Ty3*/*gypsy*-like retrotransposon widely existing in *C. albicans*. It has 277bp LTRs and contains a little part of a coding region.

There are two major data sets of genome sequence have been determined for *C. albicans* strain SC5314.[43] The first is Assembly 6 and another is Assembly 19.

Assembly 6 is a high coverage sequence (~10-fold) in the genome. The database has two composites called as *Tca3*Δ,[44] sharing ~99% identity with each other. Both of them are ~4.55 kb, having an internal region flanked by LTRs (**Fig. 7A**). The left LTR of *Tca3*Δ*6–2328* has the same sequence as the right LTR of *Tca3*Δ*6–1874*, higher than its right LTR (99.4%); the right LTR of *Tca3*Δ*6–2328* has 99.7% similarity to the left LTR of *Tca3*Δ*6–1874* than its left LTR (99.0%).[44] **Figure 7A** also shows that in the internal regions of both *Tca3*Δ elements there are extensive ORFs. The internal region of *Tca3*Δ*6–2328* contains one ORF almost whole length of itself, and in *Tca3*Δ*6–1874* a frameshift ORF locate in the internal region. These ORFs contain the RT, RH and IN domains like *Ty3*/*gypsy* retrotransposons in sequence.

Assembly 19 represents an initial draft of the diploid genome, sharing a similar *Tca3* complement to Assembly 6.[44] However, it has some information which Assembly 6 does not contain, such as a composite on contig 19–10140.[44] Assembly 19 contains identical

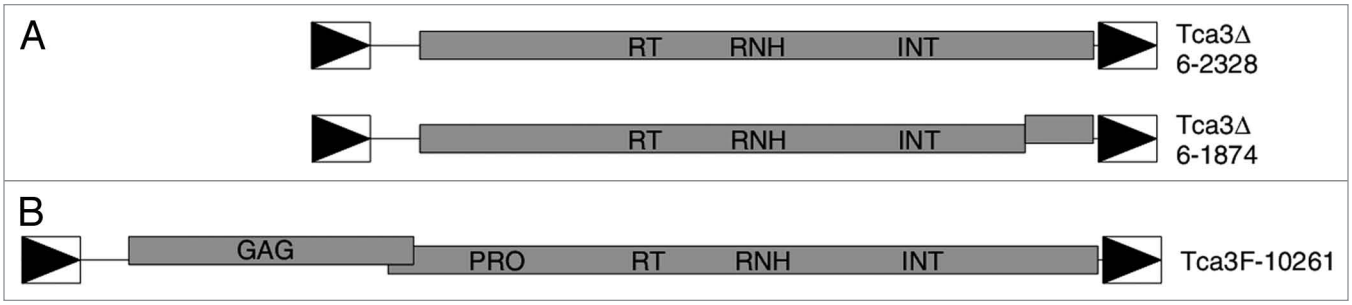

Figure 7. Structures of *Tca3* elements. (**A**) The two *Tca3* elements in Assembly 6 of the Stanford *C. albicans* sequencing project database. (**B**) A full-length *Tca3* element from *C. albicans* strain ATCC10261. In all panels shaded boxes represent the ORFs of the elements. The locations of the conserved domains are indicated. Offset boxes and vertical lines within the boxes represent frameshifts and premature stop codons, respectively. The LTRs are represented by the boxed triangles. The common scale is shown at the bottom.

LTRs flanking a target site duplication instead of the internal region. Data suggest that the sequence of internal region is substituted by a run of Ns. Highly similarity to other *Tca3* elements may lead the internal sequence co-assemble with them mistakenly.

Tca3 is roughly the same as other *Ty3*/*gypsy* elements in structure except for some special features. For example, elements of *Tca3* seem to have a distinct mechanism for DNA synthesis which is involve in minus-strand DNA. Second, elements have a distant relationship with other *Ty3*/*gypsy*-like elements. Third, the ORFs of *Tca3*Δ elements have no obvious *pol* domain and shorter RT domain as typical *Ty3*/*gypsy* elements. Finally, losing the *pol* domain and large part of *gag* domain is another special feature.[44]

A copy of *Tca3* obtained from ATCC10261 that retains the *gag*/*pol* region is referred to as *Tca3F-10261*[44] (**Fig. 7B**). It is found that *Tca3*Δ is more common than *Tca3F*. *Tca3F* is 6134 bp long. The internal region contains two long ORFs flanking by identical 313 bp LTRs. *Tca3F-10261* has 99% identity with Assembly 6 *Tca3*Δ elements, except for the ~1.6 kb indel covering the *gag*/*pol* region, a 42 bp indel upwards to the large indel, and some variation in the 5′ untranslated regions.

Goodwin et al.[44] have found 5 of 6 strains emerged *Tca3* bands, suggesting that *Tca3* is quite common in *C. albicans*. In the five examined strains, only 1 to 3 bands were produced, which indicates that the elements are present in low copy number (data not shown). This might be the chance events in evolutionary process leading to excessive *Tca3* elements produced. It may also be the result of selection, or may have connection with asexual reproduction in *C. albicans*.[45,46] However, it is difficult to evaluate the relationship between different forms of *Tca3* and host's reproductive mode, on the base of indefinite reproduction in *C. albicans*.[47] Sequence comparison and Southern analysis have shown that *Tca3* is much conserved in sequence and structure, either in loci or in strains, suggesting that the original deletion event has occurred not long ago.

Tca4, an Active Element, Contains Intact ORF

Tca4 is a *Ty1*/*copia* element closely related to *Tca2*,[16] which is flanked by 381 bp LTRs and contains an intact *gag*/*pol* ORFs. *Tca4* is simple in sequence, suggesting that it has recently emerged. Like *Tca2*, *Tca4* is a retrotranspositionally active element that strongly depends on the growth temperature. High temperature can induce *Tca4* transcription.

Tca5, an Intact LTR Retrotransposon

Tca5 is of ~5.6 kb and 4218 bp of internal sequence flanked by identical 685 bp LTRs.[19] Only one single long ORF lies in the internal region. It is larger than most other yeast retrotransposon LTRs. Phylogenetic analysis and sequence comparison imply that *Tca5* has close connection with *Ty5* element in *S. cerevisiae*. The *S. cerevisiae Ty* LTRs range in length from 251 bp for *Ty5*[38] to 371bp for *Ty4*.[48,49] The terminal inverted repeat sequences are the most conserved in *Ty1*-like elements, containing at least 5 nt: 5′-TGTTG…CAACA-3′. Therefore, the *Tca5* retrotransposon begins with TG and ends with CA, as most retrotransposons do. Sequencing of the PCR product revealed the left LTR, which was identical to the right LTR, and these LTR are designated as *omega*.

Close behind the downstream of left LTR is PBS which is complementary to methionine $tRNA^{iMet15}$ of initiator. Upstream of right LTR is a tract enriched in purines (ATGGGGAAG) and a similar sequence (ATGGGGAGG) at position 3132 bp.[19]

The internal region contains a single and long uninterrupted ORF with a relatively low copy number, and this ORF extends from within the left LTR (382 bp) to within 60 bp of the right LTR, with no inframe stop codon or frame shift, predicting a protein with motifs characteristic of *gag*, protease, integrase, reverse transcriptase, and RNase H proteins of other retrotransposons.[19]

However, it is unclear where the ORF might initiate translation in vivo.[19] There are several possible sites,[19] all of which are ATG in sequence. The first one within the ORF (382 bp from the 5′ end of the left LTR) is the unusually deep inset within the LTR. The second is also within the LTR three codons beyond the first. The third one within the ORF is unusually far beyond the end of the LTR, the three ATG are in-phase. Besides, there are several out-of-phase ATG triplets 5′ to this candidate.[19] Southern analysis (data not shown by original authors)[19] demonstrated that *Tca5* is a low copy number retrotransposon, with very few solo LTRs. It is possible that these elements contribute to the

heterogeneity of the *Candida* genome, either by recombination between the LTRs of one element or by chromosomal rearrangements between two elements. *Tca5* may still be active which is supported by the detection of full-length transcript in northern blot.[19]

In *C. albicans*, *Tca5* is the only element showing significant similarity to *Ty5*.[19] It is a suitable candidate for comparisons because it has all the expected structures of an active element and is probably intact. Comparative genomic analysis between *Tca5* and *Ty5* elements will be helpful in understanding them.

LTR-Retrotransposons Improve Genetic Diversity

C. albicans is a diploid organism.[50] Clinical isolates of *C. albicans* show variform phenotypic characteristics associated with infection and virulence.[51-57] These complex phenotypes suggest genetic diversity. However, *C. albicans* is an asexual fungus; it must have other mechanisms to generate various genomes. Retrotransposons have contributed to the evolution of genomes and genes in ways that go well beyond simply increasing genome size. It also affects genomes on a small scale, such as mobilization and integration at a locus directly, or recombination with heterotopic element indirectly. These changes contain gene inactivation, variance in transcription ability of gene, gene deletions and inversions.[29] It has been well documented that retrotransposons would mobilize in response to stress.[58-60] Servant et al.[61] have shown that the relationship between transposable activity of *Ty1* LTR retrotransposons and genome expression in *S. cerevisiae.* It seems to have a connection between stress reaction and the diversity of retrotransposons in *C. albicans*. Many organisms have the ability to recruit LTR-retrotransposons as alternative exons or promoters to drive genome evolution.[62-65] The LTRs of *Ty* elements have the ability to modify genome transcription[61] and transposable elements can influence the expression of neighboring host genes.[61,66] In gram-positive bacteria, circularized form of *Tn* retrotransposons can promote organisms resistance to antimicrobial.[67] There is an article[33] about the relationship between virulence and reverse transposition of LTR retrotransposons in *C. albicans*. Take the *Tca1* for example, the expression level of *Tca1* is related to temperature, suggesting that *Tca1* may place adjacent to the temperature-dependent genes. Since *Tca1* exhibits higher expression at 25 °C than at 37 °C, *Tca1* could upregulate the expression of genes required for the establishment of infection. Furthermore, in our original research, we found the transposition phenomenon of LTR-retrotransposon in strains which are resistant to miconazole (unpublished observation). It is suggested that there is a relationship between virulence and LTR-retrotransposons.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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