Consistent association between beta DNA element and transfer RNA genes in *Candida albicans*

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Abstract

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The database of *Candida albicans* genome was screened to investigate the relationship between tRNA genes and retrotransposons. The results showed that, among 34 distinct families of LTR retrotransposons identified, only the beta element appeared to preferentially integrate near the tRNA genes. Specific site selection of the beta LTRs may have arisen from a coevolution process in which the host’s genome used to control their transposition. It is possible that, due to this coevolution mechanism, integration of the beta elements become mutualistic, although not proven, to the *C. albicans* genome by acting as a modulator in tRNA gene transcription.

Key words: beta, LTR retrotransposon, tRNA gene, Candida albicans genome

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Retrotransposons (or retroelements) are a class of mobile genetic elements capable of transposition via RNA intermediates (Boeke et al., 1985). In eukaryotic genomes, these retrotransposon elements are abundant and ubiquitous. For instance, the human LINE1 retrotransposons make up ~17% of the human genome (Kazazian and Moran, 1998) while more than 50% of the genome of maize (Zea mays) are identified as these elements (SanMiguel et al., 1996). All retroelements exhibit two unique features: i) ability to transpose within the organism’s genome and ii) ability to replicate their copy number autonomously. Therefore, due to these properties, the retrotransposons are expected to play a key role in genomic rearrangements and thus yield an important consequence in genetic evolution.

LTR elements are the most numerous classes of retrotransposons. They generally contain two long terminal repeat sequences (LTRs), typically ranging from 250 – 600bp in length, flanking a protein-coding product with structural homology to retroviral gag- and pol-proteins (Boeke, 1989). However, the LTR retrotransposons do not have the env gene of the retroviruses. These LTR retrotransposons can be divided into two groups based upon the order of their protein-coding domains of the pol-gene unit and referred to as the gypsy-like and copia-like retrotransposons. Full-length LTR retrotransposons seem to be unstable and, for most cases, they are found in single, isolated form, the so-called “solo LTRs”. The existence of the solo elements is believed to occur from recombination between their two LTRs with subsequent loss of the internal sequence. Both full-length retrotransposons and the solo LTRs are often found flanked by the short direct repeats (4 – 5bp), a characteristic footprint of retrotransposons (Roeder and Fink, 1980).

Retrotransposons can theoretically insert into many different sites in the organism’s genome and, as such, are a common cause of genetic variation. Even worse, such a transposition may lead to a deleterious effect on host survival if some crucial genes were disrupted and thus nonfunctional. Surprisingly, the magnitude and successful dispersal of retrotransposons in the organism’s genome seem not to affect the fitness of the host, however. Detailed analyses have shown that most retrotransposons exhibit a target site selectivity within the genome suggesting the non-random behaviour of the retroelement insertions (Zou and Voytas, 1997; Kim et al., 1998; Singleton and Levin, 2002). For example, all the Ty (Ty, transposon in yeast) elements designated as Ty1 to Ty5 of the budding yeast Saccharomyces cerevisiae
have a strong bias for target site selection in the genome. Ty1, Ty2, Ty3, and Ty4 elements are found within 750bp upstream of tRNA genes or other genes transcribed by RNA polymerase III (Kim et al., 1998) whereas Ty5 targets the telomeres and mating type loci (Zou et al., 1996; Zou and Voyer, 1997). In addition, two retrotransposons, namely DRE and Tdd3, in a soil-living amoeba Dictyostelium discoideum also have the target site preference to the tRNA genes (Marschalek et al., 1992; Marschalek et al., 1993). The mechanisms that these elements choose to integrate at the specific regions, however, remain unanswered.

Recently, more than 30 distinct groups of retrotransposons have been identified and characterised in the human pathogenic yeast Candida albicans (Goodwin and Poulter, 2000). These LTR retrotransposon population found in C. albicans seem to differ considerably from Ty elements of S. cerevisiae in many aspects such as the number of distinct retrotransposon families and the copy number of retrotransposons. In addition, the majority of C. albicans LTR retrotransposons appear to be inactive, i.e., they are not able to move from one place to another in the genome. In this study, the relationship between LTRs and tRNA genes in C. albicans was examined. A wide-genome survey in C. albicans indicates that only the beta DNA element appears to preferentially integrate near the tRNA genes.

Experimental approach

Sequence data for C. albicans strain SC5314 was obtained from the Stanford DNA Sequencing and Technology Centre available at the following website: http://www-sequence.stanford.edu/group/candida. Sequencing of C. albicans was accomplished with the support of the NIDR and the Burroughs Wellcome Fund.

Goodwin and Poulter (2000) have recently identified thirty-four distinct families of the LTR retrotransposons from C. albicans. The complete set of these LTR sequences, deposited in GenBank, were retrieved according to their accession numbers and were submitted individually as a query in a BLASTN search (Altschul et al., 1997) of the Stanford C. albicans genome database. The resulting contigs derived from the search were then determined if they contained a tRNA gene using the tRNAscan-SE software version 1.21 available at the following website, http://www.genetics.wustl.edu/eddy/tRNAscan-SE (Lowe and Eddy, 1997). Please note that the results presented in this study were studied until September 2003.

The contigs containing LTRs and tRNA genes were given the reference numbers (see Table 1) that is identical to those assigned by the Stanford DNA Sequencing and Technology Centre. However, it should be noted that these ID numbers are subject to change when the C. albicans genome is complete.

Results and Discussion

The genome of C. albicans is presently being sequenced by the Stanford DNA Sequencing and Technology Centre. The data, obtained from the website http://www-sequence.stanford.edu/group/candida, were used to study if there is a specific pattern of the integration of the LTRs (i.e., near the tRNA genes) in C. albicans. In this study, the C. albicans genome was screened by performing sequential BLAST searches of individual LTR sequences against the database. By using the BLASTN programme (Altschul et al., 1997), a group of contigs that were varied in numbers depending on the LTR template used were retrieved. The presence of the tRNA genes within these contigs was subsequently confirmed by use of the tRNAscan-SE (Lowe and Eddy, 1997).

Among 34 distinct LTR retrotransposons investigated, only eight families were found associated with the tRNA genes (see Table 1). Considering that C. albicans is closely related to S. cerevisiae (Lloyd and Sharp, 1992), the results obtained were quite surprising since only a minority group of C. albicans LTRs was tRNA gene-related. In S. cerevisiae, all Ty1 – Ty4 elements (especially Ty1 and Ty3) preferentially insert within 750bp upstream of the tRNA genes (Kim et al., 1998). Besides, for most C. albicans tRNA gene-associated LTRs, the insertion bias
Table 1. The tRNA gene-related LTR retrotransposons in Candida albicans

<table>
<thead>
<tr>
<th>LTR element</th>
<th>Contig number</th>
<th>tRNA gene(s)</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha (10)</td>
<td>6-2474</td>
<td>Pro-ttRNA\textsubscript{TGG} (i)</td>
<td>808</td>
</tr>
<tr>
<td>beta (10)</td>
<td>6-2165</td>
<td>Asp-ttRNA\textsubscript{GTC}</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>6-2041</td>
<td>Asp-ttRNA\textsubscript{GTC}</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>6-2409</td>
<td>Val-ttRNA\textsubscript{AAC}</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>6-2419</td>
<td>Glu-ttRNA\textsubscript{TTC} (i)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>6-2302</td>
<td>Arg-ttRNA\textsubscript{TCT}</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>6-1799</td>
<td>Thr-ttRNA\textsubscript{AGT}</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>6-1986</td>
<td>Gly-ttRNA\textsubscript{GCC}</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>6-2469</td>
<td>Ala-ttRNA\textsubscript{AGC}</td>
<td>13</td>
</tr>
<tr>
<td>zeta (19)</td>
<td>6-2510</td>
<td>Phe-ttRNA\textsubscript{GAA} (i)\textsuperscript{d}</td>
<td>— \textsuperscript{f}</td>
</tr>
<tr>
<td></td>
<td>6-2510</td>
<td>Val-ttRNA\textsubscript{TAC} (i)</td>
<td>669</td>
</tr>
<tr>
<td></td>
<td>6-2254</td>
<td>His-ttRNA\textsubscript{GTG} (i)</td>
<td>617</td>
</tr>
<tr>
<td>upsilon (6)</td>
<td>6-2510</td>
<td>Phe-ttRNA\textsubscript{GAA} (i)\textsuperscript{d}</td>
<td>499</td>
</tr>
<tr>
<td>psi (30)</td>
<td>6-2418</td>
<td>Ser-ttRNA\textsubscript{AGA}</td>
<td>78</td>
</tr>
<tr>
<td>kahu (17)</td>
<td>6-1837</td>
<td>Pro-ttRNA\textsubscript{TGG} (i)</td>
<td>53</td>
</tr>
<tr>
<td>rho (13)</td>
<td>6-2413</td>
<td>Leu-ttRNA\textsubscript{GAA} (i)</td>
<td>671</td>
</tr>
<tr>
<td>phi (14)</td>
<td>6-1695</td>
<td>Leu-ttRNA\textsubscript{GAA} (i)</td>
<td>251</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Of thirty-four distinct LTR retrotransposons being identified in C. albicans, only those associated with the tRNA genes are presented. The copy numbers of the LTRs, which are estimated from the C. albicans genomic database (Goodwin and Poulter, 2000), are also shown in parentheses.

\textsuperscript{b} Reference numbers of the contigs used are identical to those assigned by the Stanford DNA Sequencing and Technology Centre.

\textsuperscript{c} (i) indicates the presence of intron sequences.

\textsuperscript{d} The Phe-ttRNA\textsubscript{GAA} gene identified from zeta and upsilon from the contig 6-2510 was, in fact, identical according to the sequence analysis (see text for details).

\textsuperscript{e} Numbers of nucleotides are illustrated to show a distance between the tRNA genes and the LTR elements. The distance includes the short direct repeats (if any).

\textsuperscript{f} The 3'-region of the Phe-ttRNA\textsubscript{GAA} gene is shown to be the initial part of the zeta element.

The genomic organisation of these LTRs was then subject to further analysis regarding their location with respect to the tRNA genes. For most cases, the tRNA gene-associated LTRs (except upsilon) were found upstream of the tRNA genes. However, there were differences in nucleo-
otide distance between the LTRs and the tRNA genes which varied considerably ranging from 12bp of beta to 808bp of alpha (see Table 1). In the case of Phe-tRNA_{GAA} gene (contig 6-2510) identified from zeta, its sequence was found overlapping in which the 3’-region of the Phe-tRNA_{GAA} gene was used as the initial site of the zeta DNA element. Furthermore, two Phe-tRNA_{GAA} genes yielded from zeta and upsilon were, in fact, the same gene based on their structural gene (see Figure 2).

In the budding yeast S. cerevisiae, a close association between Ty elements and tRNA genes has been well established (Chalker and Sandmeyer, 1992; Ji et al., 1993; Kim et al., 1998). However, such a relationship is still unclear in C. albicans. According to the results presented here, it seemed that most C. albicans LTRs did not have the target site preference (i.e., near the tRNA genes). Besides, most tRNA gene-related LTRs appeared to be less specific to the integration position in terms of their copy numbers (in which a few tRNA genes could be identified) and the nucleotide distance. The only element that was apparent in target bias is beta as previously described (Perreau et al., 1997; Chukeatirote, 2002). This element, like Ty3, was always found immediately upstream of the tRNA genes within a range of 12 – 36bp. Although, this preliminary study revealed that most LTRs in C. albicans did not select the tRNA genes as a specific integration, further study should be undertaken to determine if there would be any other specific sites for LTRs’ integration. Due to the unfinished state of the C. albicans genome project, distribution of the C. albicans LTRs remained unclear. Undoubtedly, the organisation of the tRNA genes and LTR retrotransposons will be better understood with the complete nucleotide sequence of the C. albicans genome.

Retrotransposons are abundant within eukaryotic genomes. Considering the potential threat that transposition may have caused (i.e., by disrupting the essential genes), there must be a mechanism used by an organism’s genome to minimise these deleterious mutations. Several studies show that the retrotransposon’s integration is not random and usually displays a strong bias for specific regions (Zou et al., 1996; Winckler et al., 1998; Kim et al., 1998). To date, it has been pointed out that the tRNA genes are likely to be the primary determination, albeit not universal, for retrotransposon’s insertion. The mechanism of how the LTRs select the tRNA genes as their preference is unknown, however.

Until recently, it was believed that transcription of the tRNA genes was solely under control of DNA sequences internal to the transcribed...
region, the so-called “A and B boxes”. However, there is growing evidence that external DNA sequences, i.e., upstream and downstream sequences, were required for full transcriptional activity (Sprague, 1995; Huang and Maraia, 2001). The upstream regions of the tRNA genes, in particular, are required for RNA pol III by recruiting the formation of pol III transcription factor TFIIIB. TFIIIB, a multiprotein complex, plays a major role in RNA pol III-mediated transcription initiation by binding with an upstream AT-rich region (Kassavetis et al., 1995; Grove et al., 1999). The beta DNA element found adjacent upstream of the tRNA genes in C. albicans is AT-rich (more than 65%) suggesting its possible role in tRNA gene expression. Such mechanisms are likely to develop during the process of genomic evolution allowing retrotransposons to be maintained within an organism’s genome.

Acknowledgements

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References


