TY ELEMENTS OF THE YEAST SACCHAROMYCES CEREVISIAE

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ABSTRACT
Transposable elements in the yeast Saccharomyces cerevisiae are a heterogeneous group of mobile genetic elements. Ty elements transpose via an intermediate RNA-molecule using the same replication and integration mechanisms as the metazoan retroviruses. Ty1 is a retrotransposons with life cycle very similar to that of oncogenes. This similarity provides an attractive model for virus-host interaction in a genetically tractable eukaryote. Like oncogenes, responds to carcinogens with an increased formation of Ty1 mRNA. There are many articles, concerning the structure, transposition cycle and regulation, as well as post-transcriptional regulation of transposition by Saccharomyces cerevisiae Ty retrotransposons. In this review, we will observe recent advances in our understanding of structure, transposition and regulation of Ty retrotransposition in Saccharomyces cerevisiae.

Introduction
Ty elements belong to the retrotransposons group. The abbreviation “Ty” stands for “Transposons of yeast”. Saccharomyces cerevisiae retrotransposons and retroviruses are often compared because of the similarity between their life cycles and their mechanism of integrating cDNA into host genomes. Ty elements generate more copies of themselves for inserting in the host-cell genome. Ty genome contains two genes: TYA1 and TYB1, which correspond to the gag and pol genes of retroviruses, respectively (3). As with certain retroviral pol genes, TYB1 expression requires programmed ribosomal frameshifting (6).

Ty mRNA is transcribed and processed in the nucleus and then transported to the cytoplasm, where it is translated into Gag and Gag-Pol proteins (8).

The Ty elements of Saccharomyces cerevisiae produce virus-like particles (VLPs), which never leave the cell (20).

During the assembly process, Ty1 RNA is packed within the VLPs and subsequently reverse-transcribed into a full-length cDNA. In the final step of transposition the cDNA is integrated into a new site in the host genome, and the cycle can begin anew by transcribing the newly transposed element.

Structure of Ty Elements
The structure of Ty-elements is similar to that of the retroviruses, but they can be distinguished by their length, their restriction maps, the number of elements in each haploid genome, as well as by their preferred insertion sites.

Five classes of Ty elements are known so far – Ty1 (3), Ty2 (22), Ty3 (10), Ty4 (11) and Ty5 (3). Ty elements have “gag” and “pol” genes as a part of their structure, but not the “env”, therefore, they are not infective.

The highest degree of homology observed is between Ty1 and Ty 2. They are built up from an about 6 kb fragment, called epsilon (ε), flanked byLTRs (Long Terminal Repeats) (334 nucleotide sequen-
Fig. 1. Genome organization of the Ty1 elements in *Saccharomyces cerevisiae*. The conservative coding domains in the open reading frame are: RB – RNA-binding protein, PR – proteinase, IN – integrase, RT – reverse transcriptase, RH – RNase H.

The Ty4 elements are also poorly studied. They are made up of a 6.3 kb fragment, flanked by tau (τ) LTRs. Ty5 elements have so-called omega (ω) LTRs. Their integration sites are in the HMR-loci of telomeres (3) (Fig. 1).

All elements of the Ty family share common heterogeneous sequences (6), and can be divided into two general (main) types – class I and class II (16). By means of heteroduplex analysis, it has been demonstrated that these two types of elements show large non-homologous areas with different open reading frames (ORFs). Class I are the so-called Ty1-15, while class II are Ty1-17. It has been determined that these two ORFs, named TYA15 and TYA17, show considerable differences in
their DNA-sequences. However, each of them encodes a pI-protein with Mw = 50 kDa, which have different amino-acid sequences, but with a high degree of structural similarity. These pI proteins play a significant role in the biology of Ty elements (16).

Transposition of Ty Elements

There are two ways, in which Ty elements can replicate intracellularly:
- Passively, during the S-phase of the mitotic cycle, together with chromosome DNA.
- Through replication solely of the Ty element, which leads to integrating RNA in virus-like particles (VLPs) – an intermediate product of the retrotranspositional process (14, 2, 1, 23).

The life cycle of Ty-retrotransposons has several stages (Fig. 2):
- Transcription
- Translation of TYA and TYB, involving frameshifting
- Forming and maturing of VLPs
- Reverse transcription
- Integration

The Ty1 mRNA is then transported into the cytoplasm and, as a result of its translation, gives two polyprotein precursors – a Gag precursor of 49 kDa and a 199-kDa Gag-Pol precursor.

The 49 kDa Gag protein is the most abundant translational product and the major structural determinant of VLPs (21). The +1 frameshift upstream of 15 nucleotides of the Gag stop-codon occurs at the sequence CUUAGGC, which results in the synthesis of a Gag-Pol read-through protein (14).

The primary translation product Gag-49 is cleaved into two products, the 45 kDa CA-protein, which assembles into virus-like particles (VLPs), required for transposition (20), and a 4 kDa C-terminal peptide.
Ty1-encoded protein nomenclature

<table>
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<th>Systematic name</th>
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<th>Suggested name(s)</th>
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<tr>
<td>Gag (primary translation product)</td>
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<td>Gag-p49</td>
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<tr>
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<tr>
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<td>PR (protease)</td>
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<tr>
<td>IN (integrase)</td>
<td>p90, p84</td>
<td>IN, Pol-p71</td>
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Fig. 3. Ty1 processing sites. Translation of the Ty1 element yields Gag-p49 and Gag-Pol-p199 protein products, which are subsequently processed by PR. The Gag/PR processing site is present in both translational products. This site is cleaved first in the Gag-Pol-p199 polyprotein, followed by cleavage at the two downstream sites (18).

Gag-P-4, which is not required for transposition. The Gag-Pol-p199 protein also undergoes proteolytic processing and is cleaved into four proteins: CA, PR, IN and RT (see Table) (20).

As shown on Fig. 3, the PR enzymes process both Gag- and Gag-Pol translational products. The Gag-Pol polyprotein undergoes a semi-ordered cleavage by PR, releasing each of the protein components. The Gag-PR cleavage site portion is hydrolyzed first, in the same location where the smaller Gag-P-49 protein is processed. This is an essential step, and blocking this cleavage inhibits all further processing. The remaining p160-Pol polyprotein is cleaved and gives as a result PR, IN (90 kDa) and RT/RNAse H (60 kDa) (18).

Ty1’s retrotranspositional process starts with the transcription of a genome Ty1 element, catalyzed by RNA-polymerase II (4), which gives as a result one main transcript. Ty1 and Ty2’s retrotransposon RNA equals to about 1% of the total RNA in yeast (4). The RNA transcript is directly involved in the transposon’s life cycle.

The transcription-initiating site, determining a 5.7 kb long RNA synthesis, is located in a TPyGA sequence, located in the beginning of the R-region of the left LTR, and is preceded by an A-T rich sequence, resembling the TATA-box of other yeast genes (4). Termination of transcription is defined by the end of the R-region of the right LTR.

Retrotransposition of Saccharomyces cerevisiae’s Ty1 elements is temperature-sensitive. Transposition frequency is at its maximum at 22 ºC, and is still readily detectable at 30 ºC, the standard growth temperature for Saccharomyces cerevisiae. At higher temperatures transposition levels drop rapidly, and transposition is abolished at temperatures, exceeding 34 ºC (18). It was shown that the major block to transposition at high temperatures is the inhibition
of the Gag-Pol-p199 polyprotein processing, as well as a concomitant reduction of RT activity.

Ty1 cDNA-synthesis is undetectable at high temperature, which is further evidence for a virtually complete Gag-Pol processing defect. Although exogenous RT activity is not innately temperature sensitive, the RT activity in VLPs, formed at high temperatures, is greatly reduced (18).

Normally, Ty1-RNA, packed within the VLPs, is later transcribed via reverse transcription to a cDNA molecule. When initiating reverse transcription near the 5′ end of LTR-elements (in the PBS (protein-binding site)-terminating site), a short DNA molecule is produced – “a restricted minus-strand DNA”, a part of which is complementary to the 3′ end of RNA with a repeated R-region. The short “minus strand DNA” is then transferred to the 3′ end of RNA, where it acts as a primer for creating an elongated “minus-strand” (14).

The retroviral reverse transcriptases (RTs) and the LTR-retrotransposons use the host’s tRNA-molecules as primers for “minus-strand” DNA synthesis. Thus, tRNA is partially used as a retro-element (to a different degree). Ty1 and Ty3 use tRNA^Met as a primer for the synthesis of the short “minus-strand” DNA molecule. The last 10 nucleotides of tRNA^Met's acceptor stem are complementary to Ty1 PBS (14).

tRNA^Met is packed in Ty1 VLPs via an unknown mechanism. For Ty1 it has been established, that the concentration of the tRNA^Met primer in VLPs is about 10 – 40-fold greater than the cytoplasmic levels of that same tRNA.

The mechanism, through which transposon elements are transported from the cytoplasm (where Ty1 DNA-integrase is located) to the nucleus, as well as their integration in the genome, is carried out with the participation of Ty1 integrase. This enzyme has a nuclear localization signal in its C-terminus. The whole integrase molecule, together with the C-terminal fragment, is located in the nucleus (15).

Mutations in the main segment decrease in vivo retrotransposition about 50-fold (15).

It has been determined that such mutant integrase protein molecules cannot be localized within the nucleus, regardless of VLP-production and reverse transcription; wholesome integration of Ty1 in vitro has also been noted.

The integration of Ty1 elements in the Saccharomyces cerevisiae genome is a model as to how the LTR-elements “populate” the host genome without causing breakdowns in coding sequences.

Most specific of all is Ty3’s targeting mechanism. This element inserts itself at a distance of maximum four nucleotides upstream from the initiating site of the polymerase III promoter, in immediate proximity to tRNA-genes. This strategy allows Ty3 to amplify itself without the risk of damaging the coding sequences (21).

Ty1 integrates itself in an interval of 75 – 700 nucleotides upstream from the initiating site of the polymerase III (pol III)
promoter. This also prevents the coding sequences from damaging.

It is interesting that both Ty1 and Ty3 transposons – which are not similar in structure – choose the same way for integration in the genome. Ty1 and Ty5, both members of the “copia”-family, display significant differences in their target mechanisms. Ty5 is specifically inserted in regions, containing “silent” chromatin, e.g. telomere regions (9) or “silent” sequences in “mating type cassettes”.

**Regulation of Ty retrotransposition**

Ty retrotransposition frequency is very low – somewhere between $10^{-7}$ – $10^{-8}$ transpositions per cell division cycle (4). The process is controlled at both transcription and post-translation stages.

Regions, involved in the regulation of transcription in Ty1 and Ty2 retrotransposons, are the TATA-box, the 5'-end of the transposon in the U5-region, IAR (internal activation regions) – located at the beginning of the epsilon ($\varepsilon$) region (8).

Many of the classical genes also take their part in the regulation of transcription, e.g. STE-genes (sterility), which code proteins, needed for the expression of haploid-specific genes and SPT (Suppressor of transposition)-genes, a number of which are transcription factors. These genes also take part in the merging of two sex cells, as well as in the sporulation of the diploid.

Ty-elements are also controlled by the MAT-locus. It has been determined, that the level of Ty1-RNA is 20-fold lower in MAT a/α diploids. MAT-regulation controls Ty-transcription both directly and indirectly. The maximum transcription level of Ty elements has been observed in the presence of STE12-transcripts.

The SRE (Sterile Responsive Element) is located in the promoter region, to which STE12 and the transcription factor TEC1 bind. Since STE12 is repressed in the a/α diploid, the MAT control of transcription through STE12 is indirect. Ty is also under the direct control of the a/α repressor. Several sequences with partial homology to the a/α binding site of the repressor were discovered in the LTRs and in the epsilon ($\varepsilon$) regions of the Ty elements. This sequence overlaps with sites, homologous to SV-40 core enhancers (8).

Ty-transposition is also controlled in several post-transcriptional stages: translational elongation, proteolytic processing and phosphorylation (5). On the other hand, a number of post-transcriptional events depend on transposition – packing of tRNA-primers, creating a cDNA-copy in Ty mRNA, insertion of a cDNA-copy in its new location in the chromosome.

The extremely important role of the HSX1 gene for Ty1 transposition has been ascertained. Post-transcriptional regulation of Ty1-transposons has an influence on Ty1 frameshifting and is carried out with the participation of tRNA-Arg (12).

As in the case of classic retroviral pol-genomes, TYB1’s expression needs a pre-programmed ribosomal frameshifting (12). Ribosomal frame shifting solves two problems, characteristic for retroviral and retrotransposonal life cycles. The catalytic POL-protein, e.g. reverse transcriptase/ribonuclease H (RT/RH) and integrase (IN) have very low concentrations, compared to the structural Gag-protein, which plays a significant role in frame shifting and Pol-expression.

On the other hand, with the active participation of the Pol-protein, a fusion Gag-Pol-protein is created by means of frameshifting; it is also “delivered” by the POL-protein to the exact location. The TYA1-TYB1-fusion protein is synthesized by +1 frame shifting (i.e. moving the frame by one codon) in the CUUAGGC sequence. This is followed by a ribosomal pause at the rare AGG (arginine) codon and a “slip” of leucil tRNA from CUU to UUA (Fig. 4) (5).

A single copy of the tRNA-Arg (CCU) gene is located in the 10th chromosome (7). It has been proven that over-expression of
Frameshifting in Ty elements occurs in three steps. The first step necessitates recognition of the CUU codon by tRNA$^{\text{Leu}}$. Owing to the low availability of tRNA$^{\text{Arg}}$, a translational pause at the AGG codon allows time for a frameshift to occur at step 2. The commitment to frameshifting occurs at step 3 when the next +1 frame codon is recognized by its cognate tRNA (5).

The tRNA-Arg (CCU) gene decreases Ty1 frame shifting. Ty1 transposition also decreases when tRNA-Arg (CCU) level is increased (24). These genes – e.g. HSX1 – are identified as genes, taking part in heat-shock response (13).

There is only one copy of the HSX1 gene, and after destroying the hsx1 allele, the resulting mutants are still viable. However, it has also been established that Ty1 transposition is inhibited in mutants with a destroyed hsx1 allele (12).

It is proven that the mature TYB1-protein does not accumulate in mutants with missing hsx1, and the Ty ORF shift increases (12). In this way, other cell genes, taking a part in Ty1-frameshifting or in forming the Ty1-VLP-complex, can be suppressed.

A site has been found in the yeast Ty3 retrotransposon, which is responsible for translational frameshifting. This frameshifting site has some peculiarities (6). The mechanism of frameshifting is not like any other known translational shifts, which need a tRNA “slip” between alternative closely located specific codons. In the Ty3-retrotransposon, the elongation is carried out normally, with the frameshifting being stimulated by two elements, known as “hungry” codons (serine AUG), and a short RNA sequence, located in a distal direction from the GCG site. The mRNA itself can efficiently manipulate the mechanism for ORF shifting (6).

Blocking processing interferes with transposition – proteolytic processing of the TYA-TYB polyprotein is essential for transposition by Ty1 and Ty3. Mutant Ty1 elements with either short oligonucleotide insertions or deletion in their PR appeared to abolish transposition (25). Mutating a conserved active site residue of the Ty3 PR had the same effect (17). All mutants produced morphologically abnormal VLPs, containing unprocessed primary translational products. The defect of the PR mutant VLPs appears to be that cDNA synthesis is much reduced. Mutant Ty3 particles had background levels of reverse transcriptase, suggesting that processing is essential for enzyme activity (17). This was not, however, true for unprocessed Ty1
polyproteins. Though reverse transcriptase was present, endogenous cDNA synthesis was reduced to background levels (25). The VLPs contain about 10-fold less RNA either because of defective packaging, or because of degradation, though it is not clear how a 10-fold reduction could eliminate transposition (5).

For the Ty5-retrotransposon, another post-transcriptional process is characteristic – one which can regulate yeast transposition. Ty5 codes all products, necessary for the regulation of transposition, in a single ORF, but it is not capable to regulate the critical ratio between structural and enzyme products through translational changes of the ORF. In this, Ty5 is very similar to the Tf1 elements of *Schizosaccharomyces pombe* and the drosophila copia-elements (5). It is not yet clear exactly how Ty5 defines the concentration of its gag- and pol-analogues. The reason here might be alternate splicing, while for Tf1 it is known for sure that the reason is enzyme proteolysis (5).

Use of yeast genetics should allow the identification of host-encoded factors, responsible for post-transcriptional control of transposition.

The existing similarity between the molecular mechanism of Ty1 retrotransposition and that of carcinogenesis might be of significant help in explaining the role of chromatin structure in carcinogenesis. Chromatin re-modeling by carcinogens is an important part in the activation of several oncogenes. Ty1 retrotransposon is also stimulated by alterations in chromatin structure, similar to the ones found in mammalian neoplastic cells. The mutations as a result of chemical damage of DNA appear to be the major factor in carcinogenesis initiation. We have developed the Ty1 transposition assay in order to detect carcinogenic pollution in the environment.

REFERENCES