Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

inactivating vectors which produce proviruses lacking LTRs addresses this safety concern.

The delivery of retrovirus vectors into specific cell types is also required for human therapy. The envelope protein of RE viruses contains multiple binding domains and, therefore, can tolerate the extensive modification associated with the introduction of cell specific targeting sequences. Retroviruses that recognize specific target cells have been generated by the incorporation of sequences encoding antigen binding sites into the *env* gene of SNV. These targeted SNV-derived vectors are very efficient at infecting specific human cell types.

See also: **Avian type C retroviruses (*Retroviridae*); Gibbon ape leukemia virus (*Retroviridae*); Murine leukemia viruses (*Retroviridae*); Retroviral Oncogenes; Recombination of viruses; Retroviruses – type D (*Retroviridae*); Vectors: Animal viruses.**

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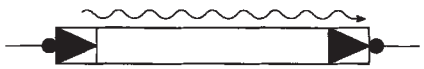
RETROTRANSPOSONS OF FUNGI



Jef D Boeke, Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, Maryland, USA

History

Transposable elements in eucaryotes can nearly all be classified into three basic types. The first type, typified by the Ac elements of plants and the P elements of *Drosophila,* resemble bacterial transposons in that they bear short inverted repeat termini; the available evidence strongly suggests that this type of element transposes directly via a DNA intermediate. However, most eucaryotic transposons differ from the bacterial elements in that they encode a reverse transcriptase (RT) or RT-like protein. Two basic types of these 'retrotransposons' are known – the LTR (long terminal repeat)-containing type, which are structurally highly reminiscent of retroviruses, and the poly(A)-type, which lack LTRs and usually (but not always) contain an oligo(A), poly(A) or similar sequence tract at their extreme 3′ end. These two types of retrotransposon are shown in **Fig. 1**. Both classes of retrotransposons are now known from organisms as phylogenetically distinct as fungi, trypanosomes, insects and mammals. Hence this brief review will focus on what is currently known about fungal retrotransposons, principally the retrotransposons that are more retroviral-like and are distinguished by the presence of LTRs. Thus, only a small and highly selective glimpse of the total picture of retrotransposons is provided.

Structural Features

The structural features of the known fungal retrotransposons are summarized in **Table 1**. LTR-containing retrotransposons isolated from fungi resemble retroviral proviruses in structure. They contain LTR sequences of a few hundred base pairs long flanking a central coding region that contains one or two open reading frames (ORFs), called *gag* and *pol* by analogy to the retroviral counterparts. As is the case with proviruses and DNA-based transposons, target site duplications of a fixed length flank the elements. These vary greatly in sequence and are presumably generated during the integration process

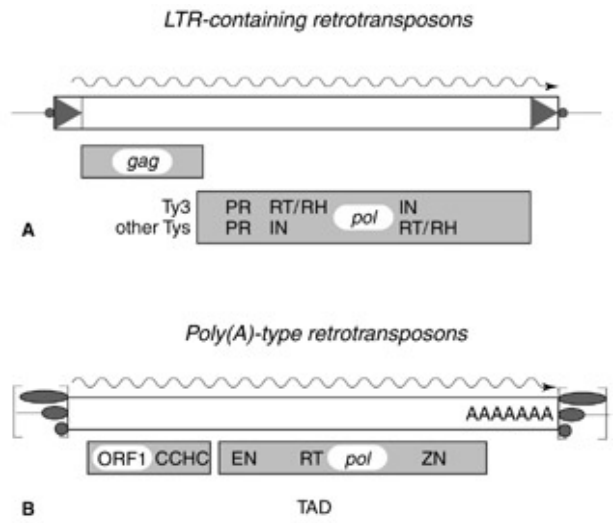


Figure 1 Retrotransposon types in fungi. All elements found to date in the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are LTR-containing transposons (**A**). PR, protease; IN, integrase; RT, reverse transcriptase; RH, RNase H. Boxed triangles represent the LTRs; wavy line represents the transcript; shaded circles represent fixed-length target site duplications. Note that the order of functional domains differs between Ty3 (*Metaviridae*) and the other yeast elements (*Pseudoviridae*). (**B**) Two elements of the 'poly(A)' type have been found in the filamentous fungi (see **Table 1**). Note that unlike almost all members of this class of elements, TAD does not contain the 3′ poly(A) tract, but instead contains an AT-rich sequence. Abbreviations and symbols as above except: CCHC, retroviral Gag Zn-finger-like sequence; EN, endonuclease domain; FZN, zinc-finger-like domain found in *pol* genes of this class of elements; bracketed shaded ovals represent variable-length target site duplications.

by a transposon-encoded integrase function. In the cases where the RNA has been examined, it resembles retroviral genomic RNA in that it extends from LTR to LTR and is terminally repetitious, allowing definition of U3, R and U5 regions of the LTR sequence in a manner formally analogous to that used by retrovirologists. The elements with two ORFs clearly contain the equivalents of *gag* and *pol*, but no analogue of retroviral *env*. The elements with single ORFs, Tf1 and Tf2, (and possibly also Ty5) apparently translate a Gag/Pol fusion protein only. In all cases, the primary translation products are then cleaved into smaller final products by the element encoded aspartyl protease.

The elements with LTRs fall into two basic classes, distinguishable by the order of the functional domains in pol (see **Fig. 1**). In the class typified by Ty3, the RT domain precedes the IN domain, as it does in retroviruses. This family of elements has recently been classified as the *Metaviridae*. In the other type, typified by Ty1 and the other yeast elements except Ty3, the order of these domains is reversed. This family of elements has recently been classified as the *Pseudoviridae*.

Thus far there is only one report in the literature of a fungal retrotransposon that lacks LTRs, the TAD element from the filamentous fungus *Neurospora crassa* Adiopodoume strain, although structurally similar elements have now been isolated from other filamentous fungi. As these elements are much less retroviral-like, they are not reviewed here.

Transposition Mechanism

There is probably more known about the LTR-containing retrotransposons of fungi than about those of any other species. By far the most heavily studied elements are the Ty1 and Ty3 elements of *Saccharomyces cerevisiae*, and the Ty5 elements of the related species *Sac. paradoxus*, and the Tf1 elements of *Schizosaccharomyces pombe*. The life cycles of these

Table 1 Fungal retrotransposons

Host[a]	*Element name*	*Type*	*ORFs*	*Target site duplication (bp)*	*LTR length (bp)*	*Primer (−) strand*
Sac. cerevisiae	Ty1	LTR	2	5	334	$tRNA^{Met}_i$
Sac. cerevisiae	Ty2	LTR	2	5	334	$tRNA^{Met}_i$
Sac. cerevisiae	Ty3	LTR	2	5	340	$tRNA^{Met}_i$
Sac. cerevisiae	Ty4	LTR	2	5	371	$tRNA^{Asn}$
Sac. paradoxus	Ty5	LTR	1	5	250	$tRNA^{Met}_i$
Sch. pombe	Tf1,2	LTR	1	5	358	self-priming
N. crassa	TAD	polyA	2	14, 17	N.A.	?
M. grisea	MAGGY	LTR	2		253	
A. fumigatus	Afut	LTR	2	5	282	

[a] Generic abbreviations: *M., Magnaporthe; Sac., Saccharomyces; Sch., Schizosaccharomyces; N., Neurospora; A., Aspergillus.*

are quite similar. In contrast, except for the presence of a cytoplasmic transposition intermediate, and evidence for reverse transcription during its transposition, relatively little is yet known about TAD transposition. This is because these poly(A)-type elements are generally less well known (and retroviral analogies are uncertain at best). Thus the discussion below applies to yeast Ty elements specifically, and to LTR retrotransposons generally.

A transposon copy (usually studied in the laboratory in the form of a *GAL* promoter/Ty element fusion) produces a transcript that extends from a point in the 5′ LTR to a different, downstream point in the 3′ LTR. Thus, a terminally redundant RNA is generated. This RNA is polyadenylated and exported to the cytoplasm, where it can have two different fates; it can serve (1) as an mRNA for Gag and Gag-Pol protein products and/or (2) as genetic material for transposition.

Translation of Ty elements is somewhat unconventional. Gag is produced directly by conventional translation of this mRNA, whereas Pol is expressed as a Gag-Pol fusion protein (readthrough protein); this 'frameshifting' process is mediated at a special sequence within the region of overlap of *gag* and *pol*. Both the Ty1 and Ty3 frameshifts differ from those of more conventional retroviruses in that they are +1 frameshifts rather than −1 frameshifts, and the intrinsic mechanism used to effect the frameshift is different. In the −1 frameshifts used by retroviruses, coronaviruses, and the yeast killer double-stranded RNA virus, a 'slippery site' allows for a simultaneous slip of the ribosome during the translational step in which the ribosomal P and A sites are simultaneously occupied. Stem–loop structures or pseudoknots that are often found just downstream of the slippery site are thought to cause ribosomal pausing and perhaps even to induce the ribosome to slip backwards. In contrast, the Ty1 element appears to utilize a completely different mechanism. There appears to be no requirement for any special RNA secondary structure; rather the frameshifting appears to be sequence-mediated, because a seven nucleotide sequence from the overlap region readily confers frameshifting on a heterologous reporter gene. Ribosomal pausing, thought to be required for frameshifting, is apparently caused by limiting amounts of a rare tRNA, $tRNA^{Arg}_{CCU}$. The stalled ribosome, which unlike the retroviral case, has an empty A site, has the P site occupied by a specific $tRNA^{Leu}$ that recognizes all six Leu codons. The stall caused by low $tRNA^{Arg}$ levels allows time for the slippage event to occur; the $tRNA^{Leu}$ slips to an overlapping Leu codon in the +1 (*pol*) frame. This then exposes a Gly codon recognized by an apparently abundant tRNA in the A site, allowing translation to continue in the *pol* frame. This mechanism results in an efficiency of frameshifting that varies somewhat with context, but on average is about 10–20%.

Once sufficient Ty protein products, in the form of intact Gag and Gag-Pol readthrough proteins, are produced, an assembly process that is not yet well understood begins. What follows is a working model for this assembly process, although this is based largely on interpretations of the limited experiments that have been done on Ty assembly and by analogy with retroviral systems and models. Ty RNA is apparently selectively packaged, together with at least one specific tRNA, the primer tRNA, into a capsid initially consisting of a coassemblage of unprocessed Gag and Gag-Pol proteins. Presumably, these coassemble via Gag-Gag interactions, and these are made in such a way that the C-termini of the proteins, and the RNA, reside in the internal cavity of the virus-like particle (VLP). The aspartyl protease encoded within *pol*, presumably activated by a dimerization process facilitated by the high protein concentration involved in the assembly process, then cleaves the precursor Gag proteins and Gag-Pol proteins to their mature, presumably physiologically relevant forms. This results in a change in the morphology of the VLPs, as well as an apparent activation of the endogenous reverse transcriptase activity.

Once reverse transcriptase is activated, the initial DNA products, corresponding to the left end of the transposon are synthesized, using a cellular tRNA as primer. This primer was recently shown to be $tRNA^{Met}_{i}$ for Ty1. Eventually, a full-length double-stranded (ds) DNA is made through a series of priming and DNA strand transfer events. In the Ty5 element, the same tRNA is apparently cleaved to form a half-molecule; the 5′ half-molecule is then used to prime reverse transcription. The identity of the enzyme that effects this cleavage is unknown.

In the Tf elements of *Sch. pombe*, a different type of reverse transcriptase priming occurs – self priming. In these unusual elements, the primer for reverse transcription is a piece of the retrotransposon RNA itself. The 5′ end of the RNA folds into a complex secondary structure, and an 11 nucleotide (nt) fragment is then released, apparently by the RNase H activity associated by the reverse transcriptase. A number of other retroelements from fungi and other organisms are thought to use a similar mechanism.

The dsDNA remains associated with Gag and Pol proteins such as reverse transcriptase inside the cell. These DNA-containing VLPs (isolated as a mixture of RNA-containing and DNA-containing VLPs) have been shown to contain all the macromolecular factors needed to carry out an *in vitro* transposition

(integration) reaction. Like retroviral core particles, which have this same activity, these VLPs require only a divalent cation for activity. The Ty DNA is apparently synthesized inside VLPs inside the cytoplasm. It is interesting to consider how and in what form this DNA is delivered to the nucleus. This is a particularly interesting question to ask in yeast, because fungi, unlike mammalian cells, undergo a 'closed mitosis' in which the nuclear membrane does not break down and reform during each mitosis, but apparently remains intact. Recent experiments implicate a small basic amino acid sequence at the C-terminus of the integrase in this process. This 'nuclear localization signal' may be responsible for delivering not only the integrase but also the DNA to the nucleus.

Once the Ty DNA and Ty integrase enter the nucleus, a concomitant cleavage of host DNA and joining to transposon ends similar to that occurring during retroviral integration occurs. Ty3 is extremely selective for its integration, and apparently inserts only at the transcription initiation sites for RNA polymerase III. Ty1 also targets tRNA genes, but probably by recognizing a unique chromatin structure associated with their 5′ ends. Ty5 elements appear to target 'silenced' chromatin. All of these mechanisms tend to ensure that these Ty elements will not destroy host genes, as most of the Ty targets do not encode genes, or at least not essential ones.

Virus-like Particles: Evolutionary Vestige or Transposition Intermediates?

The presence of VLPS in Ty elements has often raised the question of the evolutionary relationship between retroviruses and LTR-containing retrotransposons. Is the VLP a degenerate leftover of some decaying retrovirus? Or are LTR-containing retrotransposons a family of modern-day descendants of the precursor of the retroviruses? Presumably all retroelements descended ultimately from a 'cellular reverse transcriptase gene' as originally proposed by Temin. This gene may be ancient, and its original product may have been the molecule that archived the genetic information of the RNA world into DNA. Examination of the spectrum of modern-day retroelements from this perspective, reveals a natural progression from the simple to the very complex, as follows: starting with a simple RT gene (perhaps represented by modern-day telomerase?), to the poly(A)-type retrotransposons, which contain a second ORF in addition to RT, to the LTR-containing retrotransposons, which have the above plus LTRs, to the simple retroviruses, which have acquired a third ORF, *env*, and on to the most elaborate of all, the lentiviruses, with multiple additional regulatory reading frames in addition to the basic three. Since all LTR-containing retrotransposons studied appear to involve a VLP intermediate (that is, all examined to date have many properties of a transposition intermediate), the implication is that, as is the case in all complex biological reactions, a structure is built to ensure (1) high local concentration of numerous macromolecules required for the reaction and (2) appropriate orientations/conformations of these macromolecules to allow the reactions to proceed appropriately. The strongest support for this idea comes from studies on Ty1, which suggest that the VLP is a direct, functional transposition intermediate. Thus, if LTR-containing retrotransposons predated retroviruses, and their VLP structure evolved in response to selection for this organizing function, it does not stretch the imagination too far to suggest that these elements were 'preadapted' for subsequent selection for infectivity. In fact, one may see such transitional forms within the *gypsy* family of LTR-containing retrotransposons, in which some family members resemble the Tys in having two ORFs, but a few elements have

Table 2 Host genes affecting Ty1

Gene name	*Normal function/product*	*Function for Ty1*	*Found by*
Transcriptional effect genes			
SPT3, 7, 8	Transcription factor	Transcription initiation	Suppression of Ty- or LTR-induced mutation
SPT4, 5, 6	Chromatin factors	Transcription initiation	Suppression of Ty- or LTR-induced mutation
SPT10, 21	Repressor and activator	Repress 3′ LTR	Suppression of Ty- or LTR-induced mutation
Post transcriptional effect genes			
IMT1-4	Translation initiation, $tRNA^{Met}_{i}$	Prime reverse transcription	Intentional mutagenesis of genes to reveal interaction
(none)	$tRNA^{Arg}_{CCU}$	Low level causes ribosome stalling, frameshifting	Search for genes which interfere with transposition when overexpressed
DBR1	Debranch intron lariats (2′-5′ phosphodiesterase)	Unknown	Search for chromosomal mutations that interfere with transposition

a third *env*-like ORF in the appropriate genomic position.

Host Functions in Retrotransposition

The Ty1 system has provided some insights into the roles of host-encoded proteins and RNAs on the retrotransposition process, and it is anticipated that many more remain to be discovered. A large number of host genes that play roles in Ty and host gene transcription have been uncovered genetically; these are called *SPT* genes because they were originally identified by mutations that *sup*pressed the effect of *T*y or LTR insertions. Although some of these affect Ty transcription, they do not affect the production of GAL/Ty mRNA. The development of sensitive assays for transposition of GAL/Tys *in vivo* has led to the identification of a number of host factors that are important for transposition at a post-transcriptional level; these are reviewed in **Table 2.**

See also: **Coronaviruses (*Coronaviridae*); Retroviruses – type D (*Retroviridae*); Yeast RNA viruses (*Totiviridae*).**

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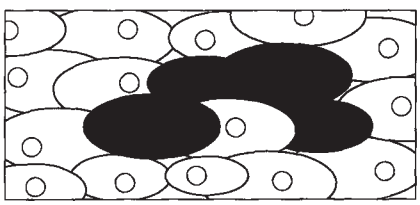
RETROVIRAL ONCOGENES



Paula J Enrietto and **Gabriela Maldonado-Codina**, Genomica Corporation, Boulder, Colorado, USA

Michael J. Hayman, Department of Molecular Microbiology and Genetics, SUNY at Stony Brook, Stony Brook, New York, USA

Introduction

The role of viruses in the etiology of cancer was suggested by the work of Peyton Rous who isolated the first acutely transforming retrovirus, Rous sarcoma virus (RSV), from a chicken sarcoma. These viruses are highly oncogenic and induce malignant transformation of cells because of the presence of cellular sequences, oncogenes (v-*onc*) within the viral genome. The cellular sequences from which viral oncogenes are derived, proto-oncogenes, are cellular genes converted into oncogenes by mutation, rearrangement or deletion. Retroviral oncogenes are generally mutated versions of their respective proto-oncogene. These changes result from the transduction process and additional changes are acquired during viral replication. Consequently, the oncogenic potential of the viral oncogene is significantly enhanced when compared to the cellular oncogene.

In this entry we will focus on retroviral oncogenes, which will be discussed in functional groups. The description of oncogenes within each group is meant to be broad. Specific details have been excluded but can be found in the references listed at the end.

Nuclear Oncogenes

ErbA

V-*erbA* is found in the avian erythroblastosis virus, AEV-ES4 and -R strains, isolated in 1934 by Englebreth-Holm. AEV transduced two different oncogenes, v-*erbA* and v-*erbB*. V-*erbB* is a homologue