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Morphogenesis at the Retrotransposon-Retrovirus Interface: Gypsy and Copia Families in Yeast and *Drosophila*

S.B. SANDMEYER and T.M. MENEES

1	Introduction	261
2	Properties of Particles	263
2.1	Copia Particles	266
2.2	Copia-like Ty Particles	267
2.3	<i>Drosophila</i> Gypsy and Gypsy-like Elements	272
2.4	Gypsy-like Ty Particles	274
3	Control of Relative Amounts of Structural and Catalytic Proteins	276
3.1	The -1 Frameshift Elements	277
3.2	Plus 1 Frameshifting of Ty1	277
3.3	Plus 1 Frameshift by the Incoming tRNA ^{Val} in Ty3	278
3.4	Regulated Translation of Major Structural Proteins from LTR Retrotransposons with Single ORFs	279
4	Priming and Reverse Transcription of LTR Retrotransposons	280
4.1	Plus and Minus Strand Primers for Reverse Transcription	280
4.2	Gypsy Gypsy-like and Ty Reverse Transcription Intermediates	282
4.3	Ty Priming by Initiator tRNA ^{Met}	283
4.4	Genetic Evidence that the tRNA Stem Is Not Copied into the Productive Plus-Strand Strong-Stop Species	284
4.5	Ty1 Replication Intermediates	285
4.6	Regulation of Ty3 Replication	286
5	Regulation of Turnover of Ty Particles	287
5.1	Turnover of Ty1 Particles in Pheromone Arrested Cells	287
5.2	Turnover of Ty3 Particles by Components of the Stress Response	288
6	Application of Particles Based on Heterologous Ty Fusions	289
7	Prospects	290
	References	291

1 Introduction

Long terminal repeat (LTR) retrotransposons have been identified in a wide variety of organisms including fungi (BOEKE and SANDMEYER 1991), plants (VOYTAS and AUSUBEL 1988; FLAVELL et al. 1995; SMYTH et al. 1989; GRANDBASTIEN et al.

1989), invertebrates (SPRINGER et al. 1991; BRITTEN 1995; BINGHAM and ZACHAR 1989), and vertebrates (FLAVELL and SMITH 1992). Many of these LTR retrotransposons can be sorted into two superfamilies named after the prototypic *Drosophila* elements copia and gypsy (mdg4). The early history of the characterization of these elements has been reviewed elsewhere (BOEKE and CORCES 1995; BINGHAM and ZACHAR 1989). Expression of representative elements results in the formation of intracellular particles analogous to the retroviral core particle. This review will focus on morphogenesis of that particle — assembly, processing, and reverse transcription — in members of these families for which the DNA sequence has been determined in the fruit fly *Drosophila* and in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces Pombe*. Collectively, these systems reveal tremendous diversity as well as striking similarities among the mechanisms members of these two LTR retrotransposon families and retroviruses have used to solve common morphogenetic problems.

Copialike and gypsylike elements are structurally similar to retroviruses. LTRs of several hundred bp flank an internal domain which contains coding information for proteins required for replication. Retrotransposons have been distinguished from retroviruses by the apparently exclusively intracellular cycles of retrotransposon replication and integration. However, this distinction has been eroded in recent years by the discoveries that retroviruses can undergo limited cycles of intracellular replication and integration (TCHENIO and HEIDMANN 1991) and that some members of the gypsylike family, and gypsy itself, encode envelope proteins (SONG et al. 1994; TANDA et al. 1994; PELISSON et al. 1994) and are transmitted extracellularly (KIM et al. 1994; PELISSON et al. 1994). Whether, for gypsy, replication is a function of extracellular transmission, as for vertebrate retroviruses, or also occurs efficiently intracellularly, as for related retrotransposons, is not yet known.

Copialike and gypsylike families are grossly distinguished by protein sequence differences in the conserved polymerase domain of reverse transcriptase (RT) (XIONG and EICKBUSH 1990; BRITTEN 1995) and in the order of the integrase (IN) and RT domains. Phylogenetic relationships of LTR retrotransposons have been described elsewhere (XIONG and EICKBUSH 1990) as have relationships of elements within the copialike (FLAVELL 1995) and gypsylike (SPRINGER and BRITTEN 1993) families. In the copialike family, the IN-coding domain precedes the RT-coding domain, but in the gypsylike family, the order is reversed. In sequence and organization, gypsylike elements are more similar to retroviruses than are the copialike elements. Given the pervasive presence of both types of elements in the plant and animal kingdoms, however, one question posed by these comparisons is why there are no copialike retroviruses. Other properties which, given their constancy in retroviruses, a priori might be expected to be consistent within either the copialike or gypsylike family, such as the mechanism by which stoichiometry of major structural proteins to catalytic proteins is maintained, the nature of the minus- and plus-strand primers, and the presence of a nucleocapsid metal finger motif are conserved between closely related elements, but not within gypsylike or copialike classes per se. Salient

sequence-derived features of the genomes of representative members of the copialike and gypsylike families from *Drosophila* and yeast for which the sequence is known are shown in Table 1.

2 Properties of Particles

Where it has been examined, reverse transcription of retrotransposons occurs associated with a particulate nucleoprotein complex (FUETTERER and HOHN 1987). The term viruslike particle (VLP) will be used to refer to the intracellular nucleoprotein particle comprising retrotransposon RNA and/or DNA and retrotransposon-encoded proteins. The term major structural protein will be used to refer to noncatalytic VLP proteins found in stoichiometric excess relative to the catalytic proteins RT and IN. The sequences of the major structural proteins of the LTR retrotransposons are not conserved, other than between very closely-related members of the copialike and gypsylike families.

Copia VLPs were characterized starting with observations in the early 1980s (FALKENTHAL and LENGUEL 1980; SHIBA and SAIGO 1983; FLAVELL 1984; EMORI et al. 1985; MIYAKE et al. 1987). Since that time, the VLPs for Ty1, a copialike element in *Saccharomyces cerevisiae* (GARFINKEL et al. 1985), and Ty3 (HANSEN et al. 1992) and Tf1 (LEVIN et al. 1990) gypsylike elements in *S. cerevisiae* and *Schizosaccharomyces pombe*, respectively, and virus particles for gypsy (PELISSON et al. 1994) have been investigated. The basic picture which emerges is that VLPs are the functional and structural equivalent of the viral core particle. Electron microscopic analysis of cells producing VLPs or of concentrated VLPs has shown heterogeneous, ovoid to spherical particles of 50 to 60 nm in size. Although observers have generally considered the core to be somewhat electron dense, populations visualized thus far have not been as homogeneous as retroviral cores, probably because they reflect multiple stages of maturation. VLPs are associated with genomic RNA as well as low molecular weight heterologous RNA species. The particles typically have a major capsid protein of 26–33 kilodaltons (kDa) and lower amounts of other proteins including: nucleocapsid, in some cases; an aspartyl protease (PR); RT/RNase H; and IN. For the purposes of this review, the abbreviations used for these proteins in retroviruses (CA, NC, PR, RT, and IN, respectively) (LEIS et al. 1988) have been adopted. Studies of virtually all of these elements have relied upon synthetic constructs or genetic systems which are conducive to high levels of expression in order to achieve preparative or readily observable amounts of VLPs. No specific subcellular site of particle nucleation has been identified for any of these retroviruslike elements. However, whether there is a transient membrane-associated phase, such as occurs in particle formation of some retroviral types, would not necessarily be known from the observations which have been made, because, as suggested above, the majority of VLPs observed in steady-state preparations are likely to be

Table 1. Properties of selected retrotransposons

Organism element (class) ^a	Element/LTR length (bp)	Inverted repeat ^b	Duplication of target site (bp)	- Strand primer binding site/primer ^c	+ Strand primer ^d	ORF/s/Gag metal finger motif ^e	gag-pol ORF-s overlap (nt) ^f	Frameshift/site ^g	Refer- ences ^h
<i>Saccharomyces</i>									
<i>cerevisiae</i>									
Ty1 (copia)	5918/334-338	TG...CA	5	ITGGTAGCGCC iMet-tRNA	GGGUGGUA	TYA, TYB/no (Gag) (Pol)	38	+1 CUU AGG C	1
Ty2 (copia)	5961/332	TG...CA	5	ITGGTAGCGCC iMet-tRNA	GGGUGGUA	TYA, TYB/no (Gag) (Pol)	44	+1 CUU AGG C	2
Ty3 (gypsy)	5351/340	TGTTGTAT...ATACAACA	5	icetGGTAGCG iMet-tRNA	GAGAGAGAGGAAGA	GAG3, POL3/yes (Gag) (Pol)	38	+1 GCG AGU U	3
Ty4 (copia)	6228/371	TGTTG...CAACA	5	ITGGCGACCCCGTAGGG Asn-tRNA	AAGGAGGCA	TYA4, TYB4/yes (Gag) (Pol)	227	+1 CUU AGG C	4
Ty5 (copia)	5375/251	TGTTGA...TCAACA	5	IGGTTATGAGCCCT iMet-tRNA fragment	GGGGGGA	1 ORF/ yes (Gag, Pol)	NA	NA	5
<i>S. Pombe</i>									
Tt1 (gypsy)	4941/358	TGTAGC...GCTaACA	5	JATAACTGAAC 5' end of Tt1 mRNA	GGGGAGGGGCA	1 ORF/no (Gag, Pol)	NA	NA	6
<i>Drosophila</i>									
Gypsy	7469/482	AgTTA...TAAT	4	TGGCGCCCAAC Lys-tRNA	GAGGGGGGAGU	ORF1, ORF2, ORF3/no (Gag) (Pol) (Env)	58	-1 AAU UUU UUA GGG	7
17.6 (gypsy)	7439/512	AgTgaCA...TgcAAT	4	TGGCGCAGTCGATGTGAT Ser-tRNA	AAGGGAAGGGA	ORF1, ORF2, ORF3/no (Gag) (Pol) (Env)	46	-1 GAA AAU UUU CAG	8
297 (gypsy)	6995/414	AGTga...TtACT	4	TGGCGCAGTCGGTAGGAT Ser-tRNA	AAGGGAAGGGA	ORF1, ORF2, ORF3/no (Gag) (Pol) (Env)	46	-1 GAA AAU UUU CAG	9
tom (gypsy)	7060/474	AGTga...TtACT	4 or 5	TGGCGCAGTCGGTAGGAT Ser-tRNA	AAGGGAAGGGA	ORF1, ORF2, ORF3/no (Gag) (Pol) (Env)	40	-1 GAA AAU UUU CAG	10
412 (gypsy)	7440/481	TGTAgT...ATACA	4	ITGGCGACCGTGACAGTCG Arg-tRNA	AAAAGGAGGGGGA	ORF3, ORF4/no (Gag) (Pol)	88	-1 GAA AAU UUU CAG	11

Ulysses (gypsy)	10653/2136	TGTT...AACA	4	JataTGCGGCCAA	GGUGA	ORF1, ORF2/no (Gag) (Pol)	32	+1	12
microplasma	5487/605	TGTCG...CGACA	4	Lys-tRNA IGAAGTGGGAT	GAATATGTCAGAAATGGCGC	1 ORF/yes (Gag, Pol)	NA	NA	13
gypsy	5146/276	TGTTG...CAACA	5	Leu-tRNA JGGTATGGGCCAG	GAGGGGGCGC	1 ORF/yes (Gag, Pol)	NA	NA	14
copla				iMet-tRNA fragment					

^a Class indicates order of *pol* gene products: copia, IN-RT/RH; gypsy, RT/RH-IN, where IN is integrase and RT/RH is reverse transcriptase/RNase H.

^b Refers to sequence at ends of LTRs. Uppercase denotes part of repeat structure; lower case denotes not part of repeat structure.

^c Uppercase denotes complementarity with primer, lowercase denotes no complementarity with primer.] indicates border with 5' LTR sequence. iMet-tRNA, initiator methionine tRNA.

^d Presumed plus strand primer = polypurine tract adjacent to 3' LTR in element's genomic RNA. [indicates border with 3' LTR sequence.

^e ORF, open reading frame. Identities of gene products encoded by ORF's are indicated in parentheses below each ORF. By analogy to retroviruses: Gag, structural proteins; Pol, catalytic proteins; Env, envelope proteins. Gag metal finger motif is a cys/his sequence of the type found in the retroviral nucleocapsid proteins, which are required for binding/packaging of retroviral genomic RNA.

^f NA, not applicable because the element has a single ORF.

^g +1 and -1 indicate the number of nucleotides that ORF2 is shifted relative to ORF1. Site is the sequence of the mRNA where the frameshift occurs. Codons indicated represent a frame. NA, not applicable because the element has a single ORF.

^h 1, CLARE and FARABAUGH (1985), BELCOURT and FARABAUGH (1990), BOEKE and SANDMEYER (1991) (and references therein); 2, WARMINGTON et al. (1985), BOEKE and SANDMEYER (1991) (and references therein); 3, HANSEN and SANDMEYER (1990), BOEKE and SANDMEYER (1991) (and references therein); 4, STUCKA et al. (1989), JANETZKY and LEHLE (1992), BOEKE and SANDMEYER (1991) (and references therein); 5, VOYIAS and BOEKE (1992, 1993), ZOU et al. (1996); 6, LEVIN et al. (1990, 1993), H. LEVIN (personal communication); 7, MARLOR et al. (1986), BINGHAM and ZACHAR (1989) (and references therein); 8, SAIGO et al. (1984), BINGHAM and ZACHAR (1989) (and references therein); 9, INOUE et al. (1986), BINGHAM and ZACHAR (1989) (and references therein); 10, TANDA et al. (1988, 1994); 11, YUKI et al. (1986), BINGHAM and ZACHAR (1989) (and references therein); 12, SCHEINKER et al. (1990), EVGENEV et al. (1992); 13, LANKENAU et al. (1988); 14, EMORI et al. (1985), MOUNT and RUBIN (1985), BINGHAM and ZACHAR (1989) (and references therein).

representative of the more prolonged stages in particle formation. Details of the foregoing observations have been presented in previous reviews (BOEKE and CORCES 1989; FINNEGAN 1989; BOEKE and SANDMEYER 1991). This discussion will primarily focus on features which distinguish particular systems or relate to recent discoveries.

2.1 Copia Particles

The copia element contains a single long open reading frame (ORF) of 4227 nucleotides (nts) (MOUNT and RUBIN 1985). The major structural proteins are encoded in a 2-kilobase (kb) RNA derived from a splicing event which joins a splice donor at position 1604 to an acceptor close to the end of the ORF (YOSHIOKA et al 1990; BRIERLEY and FLAVELL 1990; MILLER et al. 1995), creating a smaller ORF capable of encoding a protein of 48 kDa, consistent with the approximate mass of the protein previously observed to be expressed from the 2-kb transcript (FLAVELL et al. 1980). Analysis of the predicted sequence of this protein revealed a single copy of the metal finger motif found in NC in retroviruses, beginning at amino acid number 232 in the protein and an aspartyl PR-active site motif, DSG, beginning at amino acid position 292 (MOUNT and RUBIN 1985).

Transformation of clones for a complete copia element and cDNA for the 2-kb RNA into *D. hydei*, which does not contain endogenous copia elements, allowed analysis *in vivo* of the proteins encoded by the complete and spliced transcripts (YOSHIOKA et al. 1990). Both constructs sponsored accumulation of copia particles. The major protein species detected by antisera generated against VLPs is 33 kDa. *In vitro* translation of the 2-kb species showed an additional polypeptide of 23 kDa, which was inferred to be the protease based on the observation that it did not react with the antiserum which recognized the amino-terminal 33-kDa species. Based on the presumption that a mutation introduced into the putative PR-active site aspartyl residue would allow identification of the predicted higher molecular weight precursor species, a mutation was introduced, changing it to an alanyl residue. This construct was used to generate RNA, which was translated *in vitro*, and a precursor species of about 50 kDa was identified.

Copia particle formation is sensitive to the proper ratio of the major structural and putative catalytic proteins. While *in vivo* expression of the 2-kb RNA species described above resulted in accumulation of nuclear VLPs grossly similar to wild-type (wt) particles, expression of a 5-kb form of the RNA in which the 3' splice site was eliminated, but the sequence of the encoded protein was unchanged, did not result in a detectable level of particle proteins. Elimination of precursor processing also destabilized particle protein *in vivo*, as the PR-active site mutant did not generate detectable particles in the context of the 2-kb spliced construct or the full-length clone.

A particularly interesting feature of copia VLPs is that they accumulate in the nucleus. MIYAKE et al. (1987) estimated only about 2 of 500 VLPs to be cyto-

plasmic. A nuclear localization signal has not been identified, but nuclear localization of VLPs also occurs with overexpression of the 2-kb RNA in yeast where the nuclear membrane does not break down during the cell cycle (YOSHIOKA et al. 1992). This finding suggests the presence of a conserved signal for nuclear localization. Circular copia DNAs produced from reverse transcribed species represent a significant fraction of copia extrachromosomal DNA (FLAVELL and ISH-HOROWICZ 1981; FLAVELL 1984) and some of these resemble the circular copies of retroviral genomes (FLAVELL and ISH-HOROWICZ 1983). Nuclear-localized extrachromosomal retroviral DNA also accumulates as circular forms (COFFIN 1990), and the high percentage of circular copia DNA could reflect efficient nuclear localization of the particles. Circular forms of extrachromosomal copia DNA have not yet been shown to recombine into the genome at a high frequency, but they could play a role in copia genomic rearrangements by virtue of their representation in the nucleus. In yeast, recombination of reverse transcribed cDNAs of Ty1 has been observed for Ty1 IN mutants (SHARON et al. 1994).

2.2 Copialike Ty Particles

The *S. cerevisiae* elements Ty1 (CLARE and FARABAUGH 1985; HAUBER et al. 1985), Ty2 (WARMINGTON et al. 1985; FULTON et al. 1985), Ty4 (STUCKA et al. 1989; JANETZKY and LEHLE 1992), and Ty5 (OLIVER et al. 1992; VOYTAS and BOEKE 1992) are copialike in sequence and organization. Ty1 and Ty2 elements are about 5.9 kilobase pairs (kbp) in length, including 334–338-bp LTRs. These LTRs are also found as isolated insertions in the genome and are referred to as delta elements. Ty1 and Ty2 are transcribed into polyadenylated 5.7-kb RNAs beginning and ending at overlapping positions in the LTRs and containing two ORFs. The first ORF, *TYA*, is 440 codons in length and the second, *TYB*, is 1328 codons in length in a representative element, Ty1-912. The nt sequences of the internal domains of the Ty1 and Ty2 elements contain two regions of dissimilarity, the first covering much of *TYA* and the second, covering a smaller region in *TYB*. The difference in protein sequence is less, suggesting that function has been conserved.

Ty1 particle formation has been studied by fusion of sequences upstream of the transcription initiation site to strong promoters such as those for the phosphoglycerate kinase (*PGK*) (MELLOR et al. 1985b), the *GAL1* (GARFINKEL et al. 1985), and the *ADHI* genes (MÜLLER et al. 1987). Overexpression of two distinct Ty1 elements has shown accumulation of 60-nm particles (MELLOR et al. 1985b; GARFINKEL et al. 1985). More quantitative examination of large numbers of individual particles has shown that they are heterogeneous; in one study, negatively stained particles ranged from 15 to 39 nm, but unstained cryological preparations showed particles about 30% larger in diameter (BURNS et al. 1992). Electron micrographs showed spheroid to ovoid shapes and some central density, although not to the extent observed for retroviruses. Examination of several strains overexpressing Ty1 (GARFINKEL et al. 1985) showed that particles oc-

curred cytoplasmically, but in some strains hundreds of particles were collected into single clusters, while in other strains particles were isolated.

In order to identify VLP components, extracts of cells expressing high levels of Ty1 RNA and proteins have been fractionated by velocity sedimentation over sucrose or glycerol gradients. Translation of the *TYA* reading frame is predicted to produce a protein of 49 kDa. The mobility of this protein is apparently particularly sensitive to electrophoretic conditions and thus empirical estimates of the mass of this protein based on experiments in different laboratories have not been in good agreement. A protein of apparent mobility of a protein between 56 and 62 kDa (p1) present in cells expressing *TYA* alone and in cells expressing a PR mutant (MELLOR et al. 1985b; ADAMS et al. 1987a; MÜLLER et al. 1987; YOUNGREN et al. 1988) appears to be unprocessed *TYA* protein. The estimated size of the mature capsid protein processed from this precursor ranges from 47 to 55 kDa (p2) (MELLOR et al. 1985b; ADAMS et al. 1987a; YOUNGREN et al. 1988; MÜLLER et al. 1987). A smaller processing product between 5 and 7 kDa may also be present, but thus far has eluded identification. Both the *TYA* protein precursor and the processed form have been reported to have nucleic acid binding activity (MELLOR et al. 1985a,b; MÜLLER et al. 1987). Particles produced from PR mutant Ty1 elements or from *TYA* alone are more irregular in shape than wt particles. Expression of a truncated *TYA* protein designed to simulate the major processed form resulted in production of particles which were more uniform in size than wt particles and corresponded in range of sizes to the low end of that observed in wild-type (wt) Ty1 populations (BURNS et al. 1992). Incubation of immature particles formed from *TYA*-encoded p1 and artificially "matured" particles formed by expressing the truncated version of *TYA*-encoded p2 with antisera against peptides representing amino- and carboxyl-terminal epitopes, showed that amino-terminal, but not carboxyl-terminal epitopes were accessible to antibodies (BROOKMAN et al. 1995). These data are consistent with exposure of the amino-terminus of p1 on the surface of Ty1 particles, although it is possible that there are significant differences between these particles and ones formed from expressing *TYA* and *TYB*.

Wt Ty1 particles are associated with 5.7-kb Ty1 RNA, reverse transcriptase (MELLOR et al. 1985b; GARFINKEL et al. 1985), and integrase activity (EICHINGER and BOEKE 1988). The reverse transcriptase activity has been measured by both endogenous and exogenous template primer assays. Ty1 reverse transcription and transposition are temperature sensitive. In vitro reverse transcriptase activity is optimal at 20°C (GARFINKEL et al. 1985) and transposition measured in induced cultures is optimal at 18°–23°C.

Availability of the Ty1 sequence permitted directed mutagenesis of Ty1 elements in order to test the possible functions of domains of the predicted *TYB*-encoded protein. Antibodies against the putative RT domain of Ty1 showed that it was present in the particulate fraction described above and that mutations introduced into this domain abolished particle-associated RT activity (GARFINKEL et al. 1985). Small insertions and deletions (ADAMS et al. 1987a; MÜLLER et al. 1987; YOUNGREN et al. 1988) have been introduced to the region encoding PR. In cells

expressing these mutants, particles were present, but mutants differed morphologically, apparently depending on the particular element and on the mutation introduced. Generally PR mutants did not have the condensed core found in wt particles. VLPs produced from Ty1 PR mutant elements contained precursor *TYA* (56–62 kDa) and *TYA-TYB* protein (190 kDa) (p3). Interestingly, in two PR mutants, RT activity measured by exogenous assay was substantial (MULLER et al. 1987; YOUNGREN et al. 1988).

A Ty1 PR-dependent processing pathway for polyproteins into mature species has been defined using antibodies directed against specific domains encoded by *TYA* and *TYB* to analyze extracts from cells pulse-labeled with radioactive amino acid and chased with an excess of nonradioactive amino acid during expression of Ty1 (CURCIO and GARFINKEL 1992; YOUNGREN et al. 1988). *TYA* protein is processed to some extent even as early as 15 min after initiation of labeling, but processing is not complete for about 8 h. A 30-min pulse followed by a 1-h chase showed *TYA-TYB* fusion protein (190 kDa), PR-IN-RT (160 kDa), and IN-RT (140 kDa). After 24 h, mature IN (90 kDa) and RT (60 kDa) were apparent. A 23-kDa PR species was present, but was not recovered reproducibly. Mature species were quite stable and persisted in cells longer than 36 h.

Whether the heterogeneity of nonsynchronous populations of Ty1 particles can be completely explained by the presence of particles in different stages of maturation represented is not known. The finding that particles which are unprocessed or composed exclusively of *TYA* protein appear more variable, distorted, or larger than particles composed of an artificially truncated species is consistent with this interpretation. Nevertheless, the persistence of some precursor species in pulse-chase experiments suggests that even in synchronous populations, particles contain a substantial amount of unprocessed species. This is apparently particularly true for particles in cells which are not undergoing artificially elevated levels of Ty1 expression.

The system of overexpression of individual Ty1 elements has surfaced a puzzling aspect of the relationship between Ty1 expression and transposition (CURCIO and GARFINKEL 1991a). In standard laboratory haploid yeast strains, Ty1 transcripts can constitute from 0.1% to 0.8% of total RNA and, correspondingly, a substantial percentage of the poly(A) RNA. Spontaneous transposition of a marked, chromosomal element is estimated to occur at $1\text{--}3 \times 10^{-7}$ per Ty1 element per generation (CURCIO and GARFINKEL 1991b). In the case of a genetically marked, plasmid-borne, *GAL*-promoter driven element under inducing conditions, levels of the 5.7-kb RNA can be increased 220- to 225-fold. However, the transposition rate of the marked element in these cells increases by a significantly greater factor — up to 10 to 28×10^3 -fold the spontaneous level. Thus, high-level expression of a single Ty1 has a 45- to 125-fold greater effect on the transposition rate than on RNA levels. This could be explained to some extent if a significant fraction of endogenous elements are defective. However, this model was not supported by a study of a limited set of cloned chromosomal elements and a set of 39 gap-repaired Ty1 elements performed by Curcio and Garfinkel (CURCIO et al. 1988; CURCIO and GARFINKEL 1992, 1994). In the study of gap-

repaired elements, 74% were transpositionally competent. No evidence was obtained which suggested that the inactive elements had significant repressive effects on the activity of other elements. CURCIO and GARFINKEL (1992) examined steps in Ty1 particle formation and processing in order to determine at what level quantitative changes coincident with overexpression might correlate with increased transposition. Processing of the *TYA* protein precursor occurred much more efficiently in cells undergoing induction than in noninduced cells. This finding suggested that endogenous PR activity could be rate limiting in populations of cells not undergoing artificial induction of Ty1 expression. In order to test for availability of Ty1-dependent catalytic activity in populations of cells not undergoing induction of an active element, Curcio and Garfinkel tested the ability of uninduced populations to complement defects in induced, defective elements. As a control for the level of background transposition activity, they used an isogenic *spt3* mutant strain which does not express Ty1 from the native promoter and so can have no complementing activity. This study showed that a PR mutant and an IN mutant were not complemented by endogenous expression but that two RT mutants and one IN mutant were complemented and transposed at a frequency 20- to 30-fold higher if endogenous elements were expressed. Thus, they argue that overexpression of a specific, active element results in higher levels of transposition because of complementation of endogenous elements with limiting, possibly *cis*-acting processing activity. In the later study, however, CURCIO and GARFINKEL (1994) demonstrated that expression of elements results in *trans* as well as *cis* mobilization. Therefore it is unlikely that the effect of overexpression of a single element is exclusively due to saturation of a *cis* requirement for PR. Three observations may shed additional light on the nature of the limitation on processing activity. First, PR activity may be required in higher levels than is reverse transcriptase or integrase. This is suggested by the observation that some retroviruses, avian leukosis and sarcoma virus and mouse mammary tumor virus (COFFIN 1990), as well as at least one retrotransposon, copia (BINGHAM and ZACHAR 1989), express much higher levels of PR than either RT or IN. Second, in retroviruses PR must dimerize to be active, and this is likely to be the case for the Ty1-PR as well. Thus, if elements with PR defects exist, even low-level formation of heterologous PR dimers could exert a *trans*-dominant negative effect on processing. Third, electron micrographs of cells expressing Ty1 PR mutants show that particles have aberrant morphologies and appear cracked. It is possible that processing must occur within a certain kinetic window in order for particle formation to occur productively. Thus processing could be more sensitive than other steps to limiting catalytic activity. In summary, probably several factors contribute to the discrepancy between RNA levels and transposition activity. Among these are the presence of some inactive elements and a threshold requirement for proteolytic processing activity.

The Ty1 5.7-kb RNA is found associated with wt Ty1 particles (MELLOR et al. 1985b; GARFINKEL et al. 1985) and with the artificial particles produced by a truncated *TYA* protein (BURNS et al. 1992). In the latter form, it is at least partially protected against benzonase, an endonuclease of 30 kDa, but not against the

smaller 13.7-kDa RNaseA (Burns et al. 1992). This prompted Burns et al. to propose that the VLP contains the RNA within an open, cage-like structure. However, the extent to which these uniform particles lacking *TYB* proteins simulate naturally mature particles has not yet been established. Genetic assays in which one Ty1 contributes a *TRP* "donor" transcript with the *cis*-acting determinants to be reverse transcribed into DNA and integrated and a second Ty1 provides element-encoded proteins, but is not competent for reverse transcription, have been used to define the Ty1 *cis*-acting determinants required for transposition (Xu and Boeke 1990b). The smallest donor Ty1 elements found to transpose efficiently were expressed as RNAs having R-U5 and 285 nt of 5' internal sequence on the 5' end and 23 bp of internal sequence and U3-R on the 3' end. A construct with 120 nt of 5' internal sequence displayed an order of magnitude less transposition. Biochemical analysis of the composition of different helper-donor particles belied the straightforward nature of these genetic results. Although RNAs with less than 285 nt of internal sequence were not associated with Ty1 particles, analysis of *TRP1* and *HIS3* showed that these RNAs expressed under the *GAL1* promoter with no Ty1 sequences were VLP associated as was the RNA for the ribosomal protein RP51 expressed from its native promoter. These RNAs were not present in the particulate fraction when no Ty1 particles were produced, and so they were likely to have been associated with the particles. Because VLPs are not ultimately segregated in virions, however, it has been difficult to discriminate effectively between specific packaging of replication template and fortuitous particle association. Other transcripts present at high level, such as the *GAL1* transcript and transcripts encoding actin and pyruvate kinase, did not occur in association with Ty1 VLPs. Thus, the Ty1 sequence may include both positive and negative determinants of packaging, and it is possible that the *TRP1* RNA actually contributed sequences required for packaging of the Ty1 donor element. Because the number of genomic Ty1 RNAs included per particle is unknown, whether RNAs associated with the deficient donor RNA could also facilitate packaging is unclear. If dimerization occurs and is completed after particle formation, as is the case for retroviruses (Coffin 1990), this would be unlikely.

Much less is known about two other types of copialike elements, Ty4 and Ty5. The Ty4 element has sequence and organizational similarity to Ty1 and Ty2 (Janetzky and Lehle 1992). The LTRs of this element are 371 bp in length and as isolated insertions are referred to as tau (Stucka et al. 1989) elements. The Ty4 *TYA4* frame could encode a protein of 414 aa, but this protein has not yet been identified. *TYB4* overlaps *TYA4* in the +1 frame and is 1465 codons in length. One copy of the NC metal finger motif is represented in the carboxyl-terminal region of the predicted *TYA4* protein.

Ty5 was identified in the region of the type X subtelomeric repeat in the sequence of chromosome III (Oliver et al. 1992; Voytas and Boeke 1992). The element originally identified contained recognizable NC, PR, and RT-RNaseH motifs which placed it in the copialike class. A transpositionally active element has now been identified (Zou et al. 1996), so information concerning the encoded protein species should be forthcoming.

2.3 *Drosophila* Gypsy and Gypsylike Elements

The *Drosophila* gypsy and gypsylike elements for which the sequence is known include 17.6 (SAIGO et al. 1984), 297 (INOUE et al. 1986), tom (TANDA et al. 1994), 412 (YUKI et al. 1986), and gypsy (MARLOR et al. 1986). The 17.6, 297, tom, and gypsy elements contain three ORFs, as does the related element transposable element D (TED) from the moth *Trichoplusia ni* (FIESEN and NISSEN 1990). The first ORF of each of these elements encodes a putative Gag-like protein. Proteins of 15, 23, 30 and 34 kDa have been observed binding to nucleic acids and occurring in the particle preparations from cells producing gypsy (FALKENTHAL and LENGUEL 1980), but the derivation of these species has not been determined. The sequences of the proteins predicted from the first ORFs of 17.6, 297, and tom show that they form a gypsylike subfamily (INOUE et al. 1986; TANDA et al. 1988; SPRINGER and BRITTEN 1993). The second ORF of the *Drosophila* gypsy and gypsylike elements encodes proteins with PR, RT/RNaseH, and IN sequence motifs and overlaps the first ORF in the -1 frame. Less is known about these proteins than is known about the proteins encoded by Ty1 and Ty3, although RT activity is associated with gypsy virions and particles (TANDA et al. 1994). The predicted proteins encoded in the third ORFs contain hydrophobic, potentially membrane-spanning regions and appear to constitute envelope proteins.

A distinguishing feature of retroviruses when compared to retrotransposons was believed to be the obligatory retroviral cycle of extracellular infection. An important physical feature which correlates with this capacity for infection is the presence of an envelope protein. In retroviruses this ORF is expressed from a spliced mRNA (COFFIN 1990). If envelope proteins are expressed from gypsy, that implies an extracellular phase in the life cycles of these elements. SYOMIN et al. (1993) reported particles larger than typical VLPs in the culture supernatants of *D. melanogaster* and *D. virilis* cells and the presence of gypsy RNA and DNA. Other workers have gathered genetic evidence for extracellular infection by gypsy. The *ovo* gene is a hotspot for gypsy insertion, and gypsy insertion into the antimorph allele, *ovo*^{D1} (which causes sterility in a heterozygote), can revert the mutant phenotype. KIM et al. (1994) exploited this assay to demonstrate that injection of egg plasma from embryos of a strain with actively transposing gypsy elements into embryos of a strain not containing gypsy elements "contaminated" recipient embryos at a low level so that they tested positive by the reversion assay for the presence of active elements. In a second set of experiments, developing flies were fed homogenates of pupae from strains which were active or inactive for gypsy transposition. Flies fed on active strain extracts acquired genomic elements.

The data described above supporting extracellular infection by the gypsy element together with the existence of the third ORF suggested that an envelope function might be associated with gypsy particles. PELUSON et al. (1994) showed that expression of a spliced 2059-nt RNA not containing nts 569-5550 encoding the putative 482-aa (54-kDa) envelope protein correlated in follicle cells

of young, homozygous *flam* females with gypsy germline transposition activity. Monoclonal antibodies against the putative envelope protein were used to show that this species was present in tissues expressing the 2.1-kb RNA. The predicted protein has the features of an envelope protein, including an amino-terminal 13-aa hydrophobic signal sequence, a potential dibasic cleavage site, RSRR (which is the same sequence as the human T-cell leukemia virus envelope cleavage site producing surface and transmembrane proteins), and a 23-aa hydrophobic putative transmembrane domain in the carboxyl-terminal region. Song et al. (1994) used monoclonal antibodies against the predicted protein to demonstrate the presence of a glycoprotein of approximately the expected size.

The nature of the gypsy particle was also investigated in extracts from transposition-permissive flies (Song et al. 1994). Sucrose gradient fractionation showed several peaks of reverse transcriptase activity. One of these occurred at a position of lower density in the gradient than the Ty1 VLP control. This fraction showed incorporation of radioactive dNTPs into gypsy sequence in an endogenous RT assay. Examination of this fraction by immuno-electron microscopy showed irregular particles of about 100 nm which reacted with antibodies to a recombinant envelope protein. Using the *ovo*^{D1} reversion assay for gypsy mobilization described above, these investigators showed that flies not containing active gypsy elements and exposed as larvae to the appropriate sucrose gradient fractions gave rise to *ovo*^{D1} revertant progeny. At least some of these events were caused by gypsy insertions into *ovo*^{D1}, although a significant number of reversion events in one experiment were linked to copia elements. These experiments and those described above showed that gypsy particles could be transmitted extracellularly, that the presence of an envelope protein correlated with the ability of the element to mobilize in vivo, and that the features of the envelope protein are in good agreement with those expected for a retroviral envelope protein. Thus, gypsy is a retrovirus. Whether gypsy also transposes efficiently intracellularly in tissues which do not produce envelope protein and thus is also a "facultative" retrotransposon has not yet been determined.

The tom transposable element of *D. ananassae* also contains a third ORF. In this case, the genomic 6.8-kb RNA is spliced to form a 2.8-kb RNA lacking nts from position 1276 to position 5159, 16 bp downstream from the beginning of ORF3. Tom is mobilized at high frequency in the germ lines of females from the *ca; px* strain. A comparison of the expression patterns of tom RNA in the germlarium of this strain and in the related nonpermissive *ca* strain showed that the active strain accumulates only about threefold higher levels of both spliced and unspliced RNAs, but the permissive strain has high levels of tom RNA in stem cells, cystoblasts, and oocyte nuclei where it is not found in the nonpermissive strain. The predicted protein has a mass of 70.7 kDa and, if processed to remove a leader of 157 aa encoded in the first ORF, would yield a protein of 52.4 kDa. Cleavage at a basic sequence within this protein would produce a protein of 32.5 kDa and a protein of 19.9 kDa containing a hydrophobic transmembrane domain. Immunoblot analysis using antibodies against a recombinant protein based on the third ORF demonstrated reactive proteins of 59 and 42 kDa in extracts of

permissive flies. Thus, tom represents a second member of the gypsy family which has a putative envelope protein and viral properties.

2.4 Gypsylike Ty Particles

The gypsylike elements are represented in *S. cerevisiae* by Ty3 (HANSEN et al. 1988) and in *Schizosaccharomyces pombe* by Tf1 and Tf2 (LEVIN et al. 1990). Ty3 contains two overlapping ORFs. The first ORF, designated GAG3, in order to indicate its organizational and sequence differences with the other Ty elements and similarity to retroviral gag, encodes a predicted protein of 290 aa. There is a partial copy of the major homology region (MHR) motif (QGX₂EX₅FX₃L) and one copy of the NC metal finger motif CX₂CX₄HX₄C. The second ORF, POL3, overlaps GAG3 and encodes the catalytic proteins PR, RT-RNaseH, and IN.

Ty3 is naturally induced in haploid cells by mating pheromones and is consequently mobilized during mating (KINSEY and SANDMEYER 1995). In order to study cells in which prolonged expression of Ty3 resulted in accumulation of VLPs, the U3 region of a Ty3 was replaced with the GAL1 upstream activating sequence and this Ty3 was cloned into a high-copy yeast vector (HANSEN et al. 1988). Cells containing the galactose-inducible Ty3 construct make high levels of Ty3 proteins and undergo retrotransposition estimated at about 6×10^{-3} events/generation. Inspection of cells expressing high levels of Ty3 showed large clusters of 50-nm particles (HANSEN et al. 1992). Antibodies raised against peptides designed from the predicted GAG3 and POL3 protein sequences were used to identify the Ty3 proteins. Extracts of cells expressing Ty3, fractionated on sucrose step gradients, showed the presence of Ty3 proteins, genomic RNA, and extrachromosomal full-length DNA in the 70%-30% interface. These particles are about 156S. Examination of the proteins by immunoblot analysis showed a GAG3 precursor species of 38 kDa and a major 26-kDa species and minor 31-kDa and 39-kDa species which react with the antibody directed toward a determinant at the amino-terminal region of the predicted Gag3 protein. Analysis with an antibody against the carboxyl-terminal region of the predicted Gag3 protein showed the 38-kDa and 39-kDa species as well as a major 9-kDa and a minor 11-kDa species. Based on reactivity to different antibodies and amino-terminal sequence analysis, putative PR species of 16 kDa, RT of 55 kDa, RT-IN fusion of 115 kDa, and IN species of 61 and 58 kDa were identified.

The 26-kDa CA protein plays a central role in particle formation. Overexpression by galactose induction of a Ty3 element truncated after the CA-coding region showed that particles were formed (KIRCHNER et al. 1992) which could be concentrated by velocity sedimentation. The two-hybrid system of FIELDS and SONG (1989) was used to examine interactions within this domain (ORLINSKY et al. 1995). Expression of CA fused to the Gal4p DNA binding domain and Gal4p activation domain in cells with a copy of lacZ under control of the transcriptional activator Gal4p resulted in activation of lacZ expression, demonstrating that CA proteins interact. The MHR domain of retroviruses plays a role in

replication and in particle formation (STRAMBIO-DE-CASTILLIA and HUNTER 1992; MAMMANO et al. 1994). Mutations introduced into the Ty3 MHR motif (QGX₂EX₅FX₃L) changing the conserved glycyl residue to alanyl or valyl residues resulted in complete loss of particle formation (ORLINSKY et al. 1996). Mutations changing the conserved glutamyl residue to an aspartyl, asparaginy, or lysyl residue blocked formation of extrachromosomal DNA and in the case of the lysyl residue mutation blocked Gag3 processing. These results are similar to the loss of particle formation or loss of infectivity and replication which results from mutations in the MHR of retroviruses.

Ty3 NC contains one copy of the metal finger motif found in one or two copies in retrovirus NC proteins. Mutations in the metal finger domain of Ty3 NC resulted in low levels of particle formation and disrupted protein processing (ORLINSKY and SANDMEYER 1994). Whether loss of processing is a secondary effect of lack of genomic RNA was difficult to assess given the low level of particles present in cells expressing these mutants. These mutants could be complemented in *trans*, showing that there was no primary defect in ability of the precursor Gag3 to act as a substrate for processing. Although NC is encoded in *GAG3* and therefore expressed together with CA at high levels, there is evidence that NC is not required at equimolar levels with CA. A *GAG3-POL3* fusion mutant which transposes only at extremely low levels could be fully complemented for transposition by expression of CA in *trans* (KIRCHNER et al. 1992). In addition to its proposed scaffolding function for genomic RNA, retroviral NC has been demonstrated in vitro to have activities (melting and tRNA annealing to genomic RNA) compatible with other functions in replication. It is therefore possible that NC supplied in *cis* with RT has a specific function in reverse transcription. A *GAG3-POL3* fusion mutant was complemented in *trans* with a truncated Ty3 expressing only *GAG3*. Although an NC metal finger contributed from *GAG3-POL3* or *GAG3* was essential for transposition, the domain could be supplied from either source, demonstrating that the metal finger of NC supplied within the same polyprotein precursor as RT is not essential for priming reverse transcription (ORLINSKY and SANDMEYER 1994).

The Ty3 PR plays a central role in Ty3 particle morphogenesis (KIRCHNER and SANDMEYER 1993). The putative PR domain contains the sequence DSG, which is found in some retrovirus PR-active sites and is related to the sequence DTG, which is found in others. In order to test whether this motif was required for production of the Ty3 protein species found in the particles, the aspartyl residue of the active site was changed to an isoleucyl residue. This mutation blocked processing and resulted in accumulation of a low level of the predicted 38-kDa and 173-kDa Gag3 and Gag3-Pol3 precursor proteins. Mutating the active site seryl to threonyl residue to mimic the commonly occurring retroviral active site motif did not have a major effect on processing or on transposition. Ty3 elements with the PR-active site mutation had low levels of RT activity and did not transpose at a detectable frequency. Sequence analysis of the amino termini of the 9-, 16-, 55-, 61-, and 58-kDa species allowed them to be mapped precisely to the polyprotein precursor. Inference of the Ty3 PR processing sites from

these data showed that, as in the case of retroviral PR, the cleavage context is hydrophobic (P3 through P2' uncharged) and the P1 positions do not contain a branched beta carbon.

Analysis of cells expressing a Gag3-Pol3 fusion mutant produced by deletion of a single nt in the overlap region together with a CA protein allowed assignment of the 39-kDa and 11-kDa proteins as Gag3 and NC species, respectively, derived from processing of the Gag3-Pol3 precursor at a position close to or coincident with the PR amino-terminus (KIRCHNER et al. 1992; ORLINSKY and SANDMEYER 1994). Identification of the size and position of PR and the amino terminus of RT showed that there is an intervening region which potentially encodes a protein of approximately 10 kDa. Deletion of this region and substitution with a chimeric processing site permitted processing of PR and RT and did not produce gross anomalies in transposition of a *GAL*-driven Ty3 element (J. Claypool and S.B. Sandmeyer, unpublished results). The 115-kDa species contains both RT and IN determinants. Whether the active RT species is a heterodimer with RT and RT-IN subunits is unknown. Truncation of the carboxyl-terminal region of IN by as little as 27 residues reduces RT activity to background (KIRCHNER and SANDMEYER 1995), which suggests that, at the very least, the RT tertiary structure is dependent on intact IN for correct folding.

Tf1 and Tf2 gypsylike elements of *S. pombe* (LEVIN et al. 1990) have several intriguing structural differences (LEVIN et al. 1990) with other gypsylike family members. The Tf1 element has LTRs of 358 nt and is transcribed into a 4.4-kb mRNA. A transpositionally active Tf1 element contains a single ORF of 1340 codons (LEVIN et al. 1990; LEVIN and BOEKE 1992). Upstream of the region which can be inferred to encode PR based on the presence of the DTG motif, there is coding capability for a protein of about 260 aa, and a protein of 27 kDa has been identified which is encoded in this region (LEVIN et al. 1993). Antibodies directed against proteins encoded by different portions of the ORF demonstrate that initially a 140-kDa polyprotein is produced and that this is processed to the 27-kDa CA and other species, including a 56-kDa species containing IN motifs. In Tf1 elements with a mutation introduced into the PR-active site, the full-length polyprotein accumulates, indicating that at least some aspects of processing are dependent on the element-encoded PR.

3 Control of Relative Amounts of Structural and Catalytic Proteins

The mechanisms through which the LTR retrotransposons maintain the appropriate ratio of major structural proteins to catalytic proteins are striking in their diversity. In addition, classification based on these mechanisms is not congruent with copialike and gypsylike families except among closely-related elements.

Accordingly, the following discussion will be organized by mechanism rather than by family affiliation.

The correct balance of Gag and Pol proteins is established in retroviruses by ribosomal readthrough mechanisms — either nonsense codon suppression or -1 frameshifting from *gag* into *pol* (Jacks 1989). In the case of -1 frameshifting, a 7-nt sequence is present so that tRNAs positioned at the A and P sites of the ribosome can simultaneously slip into the -1 position while maintaining pairing at least at nonwobble positions. The motifs U UUA, U UUU, or A AAC are found where the first complete triplet indicates the position of the first 0 frame codon. A pseudoknot structure just downstream of the shift site enhances the frequency of frameshifting.

3.1 The -1 Frameshift Elements

Portions of the sequence of the overlap of the first and second ORFs of the *Drosophila* elements gypsy (AAU UUU UUA GGG) (Marlor et al. 1986), 17.6 (GAA AAU UUU CAG) (SAIGO et al. 1984), and 297 (GAA AAU UUU CGG) (INOUE et al. 1986) readily accommodate the retrovirus simultaneous slippage model and are related to known slippery sites. The sequence AAU UUU UUA GGG found in gypsy is the same as the sequence found at the frameshifting site of HIV-1 (JACKS et al. 1988). The sequence A AAU UUU C found in 17.6 and 297 contains shifting codons as the sequence of the frameshifting context for Mason-Pfizer monkey virus (JACKS et al. 1987). None of these sites has been demonstrated to function in the *Drosophila* element context, nor has a junction protein been sequenced, but frameshifting activity seems reasonable to suppose based on the retrovirus precedents. This mechanism is also used in the yeast L-A dsRNA virus, where 2% -1 ribosomal frameshifting from the first ORF (*gag*), which encodes an 80-kDa major coat protein, into the second ORF (*pol*), which encodes an RNA-dependent RNA polymerase, results in production of a Gag-Pol fusion protein. Frameshifting has been shown to occur on the sequence G GGU UUA and a pseudoknot which begins 4 nt downstream of the end of that sequence contributes to the frameshifting context (DINMAN et al. 1991).

3.2 Plus 1 Frameshifting of Ty1

In consideration of the retroviruslike frameshifting of the yeast dsRNA virus, it is ironic that none of the LTR retrotransposons in yeast appear to frameshift via simultaneous slippage of tRNAs in the ribosomal P and A sites. In the case of the copialike elements Ty1, 2, and 4, transition from TYA to TYB is into the +1 frame. Although, as in the case of retroviruses, a 7-nt sequence has been shown to be sufficient for at least a low level of shifting, the Ty1 frameshift differs mechanistically (BELCOURT and FARABAUGH 1990). The Ty1 sequence, CUU AGG C,

found in the 38-nt overlap between *TYA* and *TYB* mediates from 20% to 40% frameshifting, depending on the context. The salient features of this sequence were defined by monitoring the frameshifting activity of a cassette fused between coding sequences for the *HIS4* and *lacZ* proteins. They are as follows: (1) CUU is decoded by a tRNA^{Leu}_{UAG}, which has an unmodified U at the wobble position and which can decode all six leucine codons; (2) the +1 overlapping triplet is a leucine codon (UUA), which can be decoded by the same tRNA; and (3) AGG, the next 0 frame codon, is decoded by tRNA^{Arg}_{CCU}, which is a minor tRNA species encoded by a single gene. These features suggested that pausing prior to occupancy of the A site by the tRNA^{Arg}_{CCU} could permit +1 shifting by the peptidyl tRNA^{Leu} in the P site. That this was the case rather than retroviruslike simultaneous slippage was demonstrated by sequence analysis of the junction region in the frameshifted product which showed the sequence Leu-Gly rather than Leu-Arg, as would have been produced if slipping had occurred with tRNAs in both the P and A sites. In fact, expression of the rare tRNA^{Arg}_{CCU} species is inversely proportional to frameshifting (XU and BOEKE 1990a; KAWAKAMI et al. 1993). Nevertheless, a codon specifying a low-abundance tRNA is not sufficient to ensure frameshifting, as substitution of codons for other either naturally or artificially low abundance tRNAs for AGG did not sustain frameshifting. The 7-nt sequence described as the Ty1 frameshifting context also occurs in the 44-nt overlap of the first and second ORFs of Ty2 and in the 227-bp overlap of the first and second ORFs of Ty4 (JANETZKY and LEHLE 1992).

3.3 Plus 1 Frameshift by the Incoming tRNA^{Val} in Ty3

Ty3 synthesis of Gag3-Pol3 also depends upon a +1 ribosomal frameshift from *GAG3* into *POL3* (KIRCHNER et al. 1992). However, this frameshift differs in recoding site and in context from the Ty1 frameshift described above. Deletion and point mutagenesis of the 38-bp overlap between *GAG3* and *POL3* defined a 21-nt region which is essential for efficient frameshifting (FARABAUGH et al. 1993). The sequence at the beginning of the 21-bp region, GCG AGU U, was identified as the recoding site by sequence analysis of the frameshifted junction protein. In addition to identifying the site of frameshifting, this analysis showed that alanine (GCG) is followed by valine (GUU) in the protein product of frameshifting, thus excluding a simultaneous slippage model. Overexpression of the tRNA^{Ser}_{GCU}, which decodes the second 0 frame triplet in the recoding site, resulted in about a ten-fold reduction in frameshifting. This implies that a ribosome with the P site occupied by tRNA^{Ala} pauses prior to decoding in the 0 frame. In order to better understand the mechanism of shifting, two additional types of experiments were performed. Sixty-one other codons were substituted for GCG to determine whether anticodons which allowed weak pairing in the 0 frame were sufficient to support +1 shifting (VIMALADITHAN and FARABAUGH 1994). Codons CUU, GCG, and CCG allowed +1 shifting, but not other codons, even ones which would be predicted to be slippery based on stability of anticodon pairing in the 0 and +1

frames. These findings argue against slipping and imply a role for sequences in the tRNA^{Ala} structure outside of the anticodon. A prediction of this model is that another tRNA with the same anticodon would not produce the shift. Overexpression of tRNA^{Ala}_{UGC} mutated to CGC in the anticodon resulted in a twofold reduction of frameshifting, suggesting that this isoacceptor, although it decoded GCG, did not promote frameshifting and competed with the correct isoacceptor species. Farabaugh proposes that tRNA^{Ala}_{CGC} could, by interaction with an incoming noncognate species during ribosomal pausing, change the kinetics of EF-1 α proofreading in favour of that noncognate species, and that the structure of tRNA^{Val}_{IAC} in the +1 frame might be susceptible to this stabilization. Retroviral frameshifting contexts include a predicted RNA pseudoknot which contributes to ribosomal pausing. The nature of the 7.5-fold enhancement of frameshifting contributed by the 14 nt following the 7-nt recoding sequence in Ty3 is intriguing but is not known.

3.4 Regulated Translation of Major Structural Proteins from LTR Retrotransposons with Single ORFs

Surprisingly, copia (MOUNT and RUBIN 1985), and representatives of copialike (Ty5) (Zou et al. 1996) and gypsylike (Tf1) (LEVIN et al. 1990) families in *Drosophila* and yeast, contain single ORFs encoding both the major structural, non-envelope proteins and the catalytic proteins. In each case, elements with single ORFs have been shown to have transposition activity. The copia element is transcribed into a major 5-kb RNA species which is spliced to a 2-kb RNA species (YOSHIOKA et al. 1990; BRIERLEY and FLAVELL 1990; MILLER et al. 1995). As described above, particle formation by copia is dependent upon expression of the 2-kb RNA and processing by the protease encoded in that RNA. The surprising observation in this system is that levels of CA, which is encoded in the 2-kb transcript, are apparently not controlled at the level of splicing. In order to investigate potential translational control of the relative expression from the 5-kb and 2-kb RNAs, BRIERLEY and FLAVELL (1990) tested the expression of a beta-galactosidase-coding region fused to different positions in the copia sequence. They found that fusions of the beta-galactosidase-coding region to the 3' end of a synthetically spliced construct were expressed efficiently, but that a fusion at the same position in a construct in which the 3' acceptor site was disrupted by site-directed mutagenesis (i.e., the 5-kb transcript) did not produce equivalent amounts of protein. These investigators argued that differential expression from the 2- and 5-kb RNAs is consistent with translational regulation of the major structural and catalytic proteins.

Ty5 provides the first example of a single ORF retroelement in *Saccharomyces*. It is a copialike element originally identified in the sequence of *S. cerevisiae* chromosome III, adjacent to the type X subtelomeric repeat (OLIVER et al. 1992; VOYTAS and BOEKE 1992). However, identification of more complete elements found in *S. paradoxus* has allowed determination of the sequence of an

active element (Zou et al. 1995, 1996). The mechanism, if any, by which an excess of certain structural proteins is produced is not yet known.

Tf1 and Tf2 are two related elements from *Schizosaccharomyces pombe*. The DNA sequence of an active Tf1 element was determined and was shown to contain a single ORF of 1340 codons (LEVIN et al. 1990; LEVIN and BOEKE 1992). Tf1 may not be the only gypsylike element to contain a single ORF as the SURL element from sea urchins also contains a single ORF (SPRINGER et al. 1991), although transposition of that element has not yet been demonstrated. In the case of Tf1, early in the growth of induced cultures equivalent amounts of CA and a representative catalytic protein (IN) and low levels of DNA reverse transcription intermediates are detected. In stationary phase cultures, however, the ratio of CA to IN is about 26 : 1 and this transition correlates with the appearance of a eightfold higher level of approximately full-length DNA (Atwood et al. 1996).

4 Priming and Reverse Transcription of LTR Retrotransposons

4.1 Plus and Minus Strand Primers for Reverse Transcription

Genetic experiments with Ty1 showed transposition through an RNA intermediate and reiteration of regions present uniquely at each end of the genomic RNA at the other end in the DNA copy. Thus, retrotransposition displays the hallmarks of replication by retroviruslike reverse transcription (BOEKE et al. 1985).

Inspection of the putative plus-strand priming polypurine tract (ppt) sequences and minus-strand primer-binding site (pbs) of LTR retrotransposons, however, reveals unexpected differences with retroviruses (Table 1). The plus-strand primer is a short polypurine tract in the LTR retrotransposons. It ranges in length from 6 purines out of the 8 positions in Ty1 to 14 consecutive purines in Ty3. The pbs of copia (KIKUCHI et al. 1986) has complementarity to an internal region in a fragment of initiator tRNA^{Met}, which has been shown to function as the minus-strand primer. The putative pbs sequence of the copialike element Ty5 is consistent with priming from a similar position in tRNA^{Met} (VOYTAS and BOEKE 1993). The Ty1 (copialike) and Ty3 (gypsylike) elements prime from the 3' end of initiator tRNA^{Met} (CHAPMAN et al. 1992; KEENEY et al. 1995). The pbs for these elements, which is 10 nt in length for Ty1 and 8 nt in length for Ty3, is relatively short compared to those of retroviruses. Other retrotransposons have more substantial tracts of complementarity (see Table 1). The related gypsy elements 17.6, 297, and torn have putative pbs sequences which are complementary to tRNA^{Ser}. Gypsy and ulysses pbs sequences are complementary to tRNA^{Lys}, but the 412 and micropia members of this class are inferred to be primed by tRNA^{Arg} and tRNA^{Leu}, respectively.

The Tf1 element defines a new class of elements based on its mechanism of minus-strand priming. Inspection of the Tf1 and Tf2 sequences failed to reveal significant complementarity to a known tRNA sequence but uncovered an 11 nt inverted repeat between the region typically occupied by the pbs at the U5-internal domain junction and the 5' end of the genomic RNA. Changes in either the pbs region or the 5' terminal region blocked production of reverse transcripts but the combination of these changes designed to restore the predicted foldback structure was permissive for reverse transcription. These results raise some interesting questions about the priming molecule itself, among them whether priming is on a 3' OH created by processing close to the 5' end of one genomic RNA and is then templated by a second molecule or occurs on the 2' OH as observed for msDNA (FURUICHI et al. 1987). Inspection of a closely-related element, Cft-I, from the fungal tomato pathogen *Cladosporium fulvum* (McHALE et al. 1992), which has weak complementarity to known tRNA sequences, revealed that the sequence would be consistent with a 9-bp foldback structure between the pbs region and a region potentially occurring at the 5' end of the RNA (LEVIN 1995). The existence of this foldback-priming mechanism in retroelements is interesting because it offers concrete evidence that tRNAs are not the unique solution to the problem of priming retroviruslike reverse transcriptase. In addition, the existence of a mechanism for autologous priming in retroelements could have conferred independence of host tRNA populations and thus promoted horizontal transmission between distantly related species.

In addition to functioning as a scaffolding protein, NC has been implicated in tRNA annealing and priming (WILLS and CRAVEN 1991). Given the almost complete conservation of the metal finger motif in NC proteins of retroviruses, the broad inconstancy of this feature within either the copialike or gypsylike families is striking. One might speculate that some other feature of the priming apparatus might be conserved along with NC in the representative LTR retrotransposons (Ty3, Ty4, possibly Ty5, and copia). Yet the presence of the NC metal finger motif does not correlate with relatedness of the RT polymerase domain or the identity of the tRNA primer or even the position of the end of the primer in the tRNA. Additional alignments of RT may eventually reveal a motif conserved or function maintained which is common among elements and viruses containing a metal finger NC. Whether small NC-type proteins exist for the nonmetal finger *gag*-like proteins is also not known. Processing of a small, carboxyl-terminal domain occurs for at least one of these elements (Ty1), but nucleic acid-binding activity has also been reported for the mature form of this protein, which does not contain this domain.

The position of the internal pbs relative to the adjacent, upstream LTR is a variant feature of the LTR retrotransposons. It appears to be a primary determinant of whether trimming activity is required by IN prior to integration. In the case of Ty3, the position of the 3' end of the tRNA primer is 2 nt displaced from the internal 3' end of the upstream LTR as is the case for retroviruses. Species with 2 extra nt at the outside ends are predicted by the position of pbs. These extra nt have been shown to be present in extrachromosomal Ty3 DNA

and the 3' ends have been shown to be processed in wt, but not mutant IN VLPs (KIRCHNER and SANDMEYER 1995). The position of the Ty1 pbs, which anneals to the same primer species, initiator tRNA^{Met}, is exactly juxtaposed to the internal 3' end of the LTR. Based on the retroviral model, Ty1 minus-strand strong stop begins with the 3' nt of the U5 region of the RNA (CHAPMAN et al. 1992). Data from in vitro integration also indirectly support this positioning of the primer. Ty1 DNA with blunt ends and no extra nt acts efficiently in integration reactions, whereas DNA with a 4-nt 5' overhang does not act as an efficient donor (EICHINGER and BOEKE 1990). The copialike Ty4, gypsylike 412, and copia also juxtapose the pbs to the 3' end of U5. The putative pbs sequences of the gypsy, 17.6, and 297 elements overlap the 3' end of U5 by 1 nt. Although admittedly novel, the position of the pbs sequences of this family, which overlap the 3' nt of U5 with RNA, can be accommodated by the generic retroviral model of replication. In the case of the gypsy, 17.6, and 297 elements, the 5' end of the minus-strand strong-stop DNA species is, in theory, incomplete relative to the integrated form by one deoxyribonucleotide. Studies with HIV have shown, however, that RNaseH preferentially cleaves the tRNA primer so that the final ribonucleotide remains associated with the DNA. If this is also the case for gypsy, then this ribonucleotide could template synthesis of the 3' end of the plus strand. The 3' end is directly involved in strand transfer upon integration, while a 5' ribonucleotide at the end of the minus strand could be degraded during the subsequent gap repair process. The deduced LTR ends of the gypsy and gypsylike elements are also unusual in that they do not have the conserved TG/CA dinucleotide found at the termini of other LTR retrotransposons and retroviruses.

4.2 Gypsy, Gypsylike and Ty Reverse Transcription Intermediates

DNA species associated with VLPs have been examined for mdg1, mdg3, gypsy (ARKHIPOVA et al. 1986), Ty1 (EICHINGER and BOEKE 1988; MÜLLER et al 1991; PO-CHART et al. 1993a; CHAPMAN et al. 1992), and Ty3 (HANSEN et al. 1992; KIRCHNER and SANDMEYER 1996). Strong-stop species consistent with a retroviruslike mode of replication have been observed in each case. For gypsy expressed at steady-state levels, the level of plus-strand strong stop was estimated to be five to ten times higher than the level of minus-strand strong stop. For mdg1, mdg3, gypsy, and Ty1, treatment of a minus-strand strong-stop species with RNase results in a reduction in size consistent with removal of the putative tRNA primer. For mdg1, 3, and 4 and Ty1 and 3, the plus-strand strong-stop species is observed as a doublet. In the case of the mdg-gypsy elements the larger species correspond to the length of the LTR extended by the length of the region of complementarity with the putative tRNA primer. In the case of the yeast elements Ty1 (LAUERMANN and BOEKE 1994) and Ty3 (KIRCHNER and SANDMEYER 1996) primed by initiator tRNA^{Met}, the plus-strand strong stop is extended for 12 nts, up to the first modified base of initiator tRNA^{Met}.

4.3 Ty Priming by Initiator tRNA^{Met}

The function of initiator tRNA^{Met} in Ty1 and Ty3 replication has been investigated using a combination of biochemical and genetic strategies (KEENEY et al. 1995; CHAPMAN et al. 1992). Incubation of Ty1 VLPs with [α -³²P]dTTP resulted in labeling of a 75-nt RNA species, consistent with the size of the predicted initiator tRNA^{Met} primer plus one radioactive nucleotide. In the presence of all four dNTPs, a species of 171 nt is radiolabeled which is reduced to 96 nt by treatment with RNase. In order to demonstrate that the presence of this species is specifically dependent upon the complementarity to initiator tRNA^{Met}, a genetic strategy was used. Five nt changes were introduced into the pbs of Ty1 and shown to virtually abolish Ty1 transposition. Transposition of wt Ty1 was also greatly reduced in a strain in which the sole initiator tRNA^{Met} was supplied from a plasmid-borne copy of the tRNA^{Met} gene with the changes complementary to those introduced into the pbs and compensatory changes in the 5' stem (*imt4-9*). However, when the mutant Ty1 and tRNA gene were expressed in the same cells, Ty1 DNA production and transposition were restored. Northern blot analysis using an initiator tRNA^{Met}-specific probe showed that disruption of complementarity between the pbs and the priming tRNA did not affect inclusion of initiator tRNA^{Met} in the particle. In a separate study, POCHART et al. (1993b) showed that initiator tRNA^{Met} is concentrated about 11-fold in Ty1 particles relative to its concentration in total tRNA. Interestingly, a minor species, tRNA^{Ser}_{GCU}, was similarly represented in VLPs compared to tRNA^{Met}. This represented about a 150-fold concentration relative to its cytoplasmic representation.

Genetic manipulation of the primer tRNA gene has had two other interesting applications. Although it is straightforward to deduce the nt in the aminoacyl stem which pair with the pbs, the residues which contribute to inclusion of the tRNA in the particle and which may be involved in interactions with NC or RT are not self-evident. A strain containing deletions of the chromosomal *IMT1-4* genes and transformed with two plasmids, one carrying Ty1 and a copy of the mutated initiator tRNA^{Met} which is active in translation but does not prime wt Ty1 reverse transcription, *imt4-9*, and one carrying a mutated, tester initiator tRNA^{Met}, was used to probe these primer functions. Mutations which affected the level of this tester tRNA or which were located at essential positions for priming disrupted transposition. In the first analysis, mutations were introduced to 11 positions of the anticodon stem and loop region, which has been shown to be important for HIV RT-tRNA^{Lys} interaction (BARAT et al. 1989), and 4 mutations were introduced in the T Ψ arm, which has been shown to be important for avian RT interaction (Hu and DAHLBERG 1983). Single mismatches in two independent positions in the region which pairs directly with the pbs blocked transposition, but the effects of the mutations were reversed when compensatory mutations were introduced into the pbs of the Ty1 element tested, demonstrating that the effect was at the level of primer annealing. Interestingly, although single base mismatches were not tolerated, a G:U base pair between the pbs and the tRNA did not disrupt transposition. Mutations in the T Ψ arm at positions 50, 54, 60, and 64 reduced

Ty1 transposition. The extent of the effect depended on the nature of the individual mutation or combination of mutations, with the greatest effect being observed with changes of A54 to G or C, mutations which extended the arm structure, or changes which disrupted structure important for initiator function at positions U50:A64. Experiments in which chimeric initiator tRNA^{Met} genes between *S. cerevisiae* and *Arabidopsis thaliana* and *S. pombe* gene were tested showed that chimeras with differences from the natural primer in the D loop stem did not produce active primers. Together these experiments suggested that the domain created by interaction of the TΨC and D loops in the L-shaped tertiary structure could play a role in primer recognition or function.

Ty3 contains a pbs region with 8 nt of complementarity to initiator tRNA^{Met}. Ty3 does not transpose in the strain carrying the initiator tRNA^{Met} gene with the five compensatory mutations in the aminoacyl stem (*imt4-9*) (KEENEY et al. 1995), showing that tRNA^{Met} is likely to be the Ty3 primer. The effect of point mutations in the initiator tRNA on Ty3 transposition were also tested. Despite the differences noted above between Ty3 and Ty1 (weak similarity in RT, presence of NC in Ty3 but not in Ty1), Ty3 transposition showed a qualitatively similar sensitivity to mutations in the primer to that displayed by Ty1 (KEENEY et al. 1995). In view of the apparent functionality of G:U pairing in Ty1 priming described in the preceding section, the interactions between the initiator tRNA^{Met} and the Ty3 pbs probably extend past the 8-nt complementary tract through the next two bases which could form G:U pairs, giving a pbs 10 nt in length. However, this is still significantly shorter than the 18 nts observed for retroviral pbs sequences. The shorter tract of complementarity may be tolerated because of the lower growth temperature of yeast compared to animal retroviruses. Ty1 and Ty3 RTs are temperature sensitive even in exogenous assays, so the role of temperature in annealing has not been tested directly.

4.4 Genetic Evidence that the tRNA Stem Is Not Copied into the Productive Plus-Strand Strong-Stop Species

As noted above, where a plus-strand strong-stop species has been observed for LTR retrotransposons (mdg1, mdg3, and gypsy) (ARKHIPOVA et al. 1986) and Ty1 (MÜLLER et al. 1991; POCHART et al. 1993a) and Ty3 (KIRCHNER and SANDMEYER 1995), there are two species. The longer and predominant species found for the mdg1, mdg3, and gypsy are lengths consistent with copying of the primer tRNA through the region of complementarity to the pbs as occurs for retroviruses (TELESNITSKY and GOFF 1993). However, in the case of Ty1 and Ty3, which have pbs sequences of 10 and 8 nt, respectively, the length of the plus-strand strong stop appears to be consistent with copying of the tRNA primer up to the first modified base 12 nt into the initiator tRNA^{Met} (LAUERMANN and BOEKE 1994; KIRCHNER and SANDMEYER 1995). In the case of Ty1, this species has been shown

to hybridize to a pbs-specific probe (MÜLLER et al. 1991). Indeed, RNA-templated products have been shown to correct pbs mutations in retroviruses, suggesting that this species is extended to form the plus strand (TELESNITSKY and GOFF 1993). However, a similar pbs templating on the tRNA primer appears not to occur for the Ty elements. If the Ty plus-strand strong-stop species is transferred to the 5' end of the nascent minus-strand DNA and extended, the pbs of Ty elements would have been converted into a tract of 12 nt of complementarity with the primer tRNA^{Met}. LAUERMANN and BOEKE (1994) specifically tested for the transfer of information from the tRNA primer into the Ty1 pbs. A single-bp mutation was introduced at position 7 of the Ty1 pbs and its correction was monitored in preintegrative DNA by sequence analysis of amplified single strands and within integrated elements by selective restriction digestion. The pbs mutation was not corrected by tRNA templating in the extrachromosomal DNA or in the transposed elements. A system where information is transferred from the tRNA into the pbs in theory would allow a single RNA to template genomic replication even assuming that RNaseH activity degraded the 5' end of the RNA template for minus-strand strong-stop DNA. In the case of the Ty system if the 5' end of the RNA template for the minus-strand-strong-stop DNA is degraded by RNaseH, then the data suggest that the target of transfer of the plus-strand strong stop is a minus-strand DNA which has been copied past the pbs into the R-U5 region of overlap, necessarily on a separate RNA template. Since the Ty plus-strand strong-stop species extended into the tRNA would be unpaired for at least two bases at its 3' end, it may fail completely to function as a primer and accumulate while the form not containing 12 nt from the tRNA is incorporated into full-length plus strand. Alternatively, the unpaired 3' end of the plus strand could target it for degradation and repair templated by the pbs, rather than the tRNA-derived sequence. Ultimately, assuming degradation of the RNA template for the minus-strand strong stop, these observations would seem to argue for functional Ty genomic diploidy and corresponding increases in genome fluidity.

4.5 Ty1 Replication Intermediates

In addition to the strong-stop species described above, full-length Ty1 and Ty3 DNA is found associated with their respective VLPs (EICHINGER and Boeke 1988; HANSEN et al 1992). In the case of Ty1, significant amounts of intermediates occur as well (MÜLLER et al. 1991; POCHART et al. 1993a). Studies with two different Ty1 elements have shown that in steady-state VLP populations the major species are comprised of full-length minus strands and a 2.1-kb plus strand with its 3' end at the 3' end of the mature DNA. Mapping of the 5' end of this intermediate shows that it lies near the internal sequence TGGGTGGTA, which is also the sequence at the internal domain-U3 junction identified as the plus-strand primer (POCHART et al. 1993a). As observed for other elements and mammalian retroviruses, there are circular forms of Ty1. Circular Ty1 DNAs

recovered as plasmids (EICHINGER and BOEKE 1988) were 1-LTR circles. However, electron micrographic analysis of Ty1 species suggested that 2-LTR species might also be represented. Both studies (MÜLLER et al. 1991; POCHART et al. 1993a) identified plus-strand species extending from the upstream end of the RNA, about 0.4 kb in one case and over 3 kb in the other. These studies suggested that full-length, completely replicated, linear Ty1 DNA is a relatively minor species. Whether this is a strain-dependent phenomenon is not known. It will be interesting to determine whether an additional discrete step exists at which completion of reverse transcription is regulated.

4.6 Regulation of Ty3 Replication

Retrovirus reverse transcription is not completed until infection of new host cells. In addition, viral replication is sensitive to the state of replication of the host cell. Rous sarcoma virus, spleen necrosis virus, and Moloney murine leukemia virus do not complete reverse transcription in quiescent host cells. Similarly, although HIV can replicate in nondividing macrophage, it does not complete reverse transcription in quiescent human T lymphocytes (reviewed in MENEES and SANDMEYER 1994). In growing cells induced under a regulated promoter to high levels of Ty3 transcription, a major Ty3 DNA species is observed, which migrates as a heterogeneous species of about 5.4 kb (HANSEN et al. 1992). Because, it can be digested in central and terminal regions with restriction enzymes, it presumably represents a full-length duplex DNA species, rather than the DNA-RNA heteroduplex inferred for Ty1 based on incomplete plus-strand synthesis. However, under physiological conditions, Ty3 expression is induced by the pheromone signal transduction initiated when haploid cells are exposed to cells of the opposite mating type (BILANCHONE et al. 1993). Cells in this condition are not dividing but are arrested in G₁ prior to mating, at which time the synchronized populations fuse and S phase ensues. This context may be analogous in some respects to retroviruses in the original host cell or in nondividing cells.

It could be speculated that Ty3 would have greater potential for proliferation and consequently a selective advantage, if it transposed after, rather than before, cell-cell fusion. *MATa* cells were arrested in G₁ by treatment with α -factor (MENEES and SANDMEYER 1994). After complete arrest, cells carrying no endogenous Ty3 elements, but a high-copy plasmid with Ty3 under control of the *GAL1* UAS, were induced for Ty3 expression and transposition was measured using a polymerase chain reaction (PCR) assay. In log cells transposition occurred within 3 h of induction of Ty3 transcription. Examination of arrested cells showed that although induction kinetics were normal and particles were present, full-length DNA was not detectable. No block occurred in *far 1* mutant cells, which respond to α -factor signal transduction, but do not arrest. If cells were switched to a repressing carbon source for Ty3 expression and α -factor was removed, DNA synthesis and transposition occurred within 1–2 h. Whether the

mechanism of regulation depends upon reduced levels of dNTPs in G₁ cells or is through some direct effect on RT is not known. These findings predict that in natural populations of mating cells Ty3 particles form prior to mating but reverse transcription and integration occur in the new diploid cell. This was directly tested with a Ty3 element on a high-copy plasmid in one mating type and a selectable target plasmid in the opposite mating type. After mating, diploid cells which had undergone transposition of this type were identified by selection for cells where the Ty3 element from one parent had activated a suppressor tRNA on the plasmid target of the other parent (KINSEY and SANDMEYER 1995). Thus Ty3 undergoes what might be considered an inefficient infection cycle by virtue of sexual fusion of cells containing unreplicated particles with target cells in which reverse transcription is completed.

5 Regulation of Turnover of Ty Particles

Ty1 and Ty3 particle proteins appear to be relatively stable. For example, Ty1 proteins labeled in a 30-min pulse under steady-state conditions of Ty1 expression were processed to mature 90-kDa and 60-kDa forms which were still present after a 36-h chase (GARFINKEL et al. 1991). Ty3 proteins produced under inducing conditions are relatively unchanged in levels after 8 h of growth in glucose, a repressing carbon source (Menees and Sandmeyer, unpublished data). Despite this stability, as described below, the turnover of Ty particles is subject to modulation arising from the state of the cells.

5.1 Turnover of Ty1 Particles in Pheromone Arrested Cells

Xu and BOEKE (1991) tested the effect of α -factor arrest on Ty1 transposition in *MATa* cells. As is observed for Ty3, transposition is blocked by α -factor treatment. Cells blocked in α -factor were assayed genetically for evidence of transposition. Ty1 expression was induced in arrested cells. After a period of 9 h, cells were plated and monitored for transposition. Transposition was decreased by more than tenfold in cells arrested by pheromone treatment during induction. In order to determine the level at which transposition was affected, Ty1 VLPs were isolated and analyzed by Western, Northern, and Southern blotting. TYA proteins migrated with a mobility intermediate between the precursor form of 58 kDa and the processed 54-kDa form. Labeling with ³²P showed that TYA proteins in α -factor-treated cells were phosphorylated. In addition, loss of reactivity to an antibody with an amino-terminal epitope suggested that this protein was modified, perhaps by amino-terminal proteolysis. TYB-encoded 160-kDa and 90-kDa proteins were shown to be unstable, although this did not appear to be the case for the 60-kDa RT species. The level of DNA present in the particle was also greatly reduced.

5.2 Turnover of Ty3 Particles by Components of the Stress Response

Ty1 and Ty3 transposition is temperature sensitive. Ty1 transposition estimated from insertions of Ty at *ADH2* and *ADH4* selected by antimycin A resistance was 100-fold more frequent at 15°C than at 30°C (PAQUINN and WILLIAMSON 1988). Ty1 RT activity monitored in VLPs in vitro is maximal at about 15°C, but is barely detectable at 37°C (GARFINKEL et al. 1985), suggesting an explanation for the observed temperature dependence of transposition. Transposition of Ty3 was not grossly different between 23°C and at 30°C in a qualitative colony hybridization assay (HANSEN et al 1988), but was greatly reduced at 37°C. Ty3 RT is also temperature sensitive with an optimum of 25°C (HANSEN et al. 1992).

Recent investigation of the state of Ty3 VLP proteins and DNA in extracts of cells grown under inducing conditions at 30°C and 37°C showed that cells grown at 30°C contained similar amounts of the 38-kDa GAG3 protein and 26-kDa mature CA protein, but that cells grown at 37°C contained about one-fifth that amount of Gag3 precursor and no detectable CA protein. DNA which was readily detectable in 30°C grown cells was not detectable in the 37°C grown cells. A temperature shift experiment showed that particle turnover occurred with a half-life of only about 40 min in cells at 37°C and greater than 3 h at 30°C. These observations prompted an investigation into the nature of the temperature restriction of Ty3 transposition. A mild state of heat shock is produced by growth of yeast cells at 37°C (CRAIG et al. 1993; PARSELL and LINDQUIST 1993). In order to determine whether the stress response was responsible for the low level of Ty3 particle formation at 37°C, cells were stressed by treatment with 1.55 M methanol (PLESSET et al. 1982) or genetically in the *ssa1 ssa2* mutant background (STONE and CRAIG 1990). These cells also exhibited low levels of Gag3 precursor proteins and no mature CA, suggesting that the low level of Ty3 particle formation in cells grown at 37°C reflects the elevated levels of stress response proteins, rather than physical denaturation of the particle (MENEES and SANDMEYER 1996). Growth of *ssa1 ssa2* cells at 23°C, a condition which causes increased expression of the *SSB1* and *SSB2* genes for the cytosolic hsp70s (CRAIG and JACOBSEN 1985), rescued the Ty3 particle formation defect. It has been suggested that state of the *ssa1 ssa2* mutant represents a relative deficit of chaperone assembly function relative to stress response degradation functions (CRAIG et al. 1993). The formation of Ty3 particles at 23°C is consistent with this interpretation, although other explanations (such as increased stability of Ty3 VLPs at 23°C) are also reasonable. The observation that Ty3 is a substrate for turnover by the stress response suggests that it may also act an inducer of stress response proteins and that it is a substrate for ubiquitination. The effect of Ty3 on stress response protein production was monitored using antibodies against Hsp104p (a gift from S. Lindquist, University of Chicago). *HSP104* is readily induced by heat shock and has been shown by in vitro assay to have ATP-dependent disaggregating activity (PARSELL et al. 1994). Ty3 expression under galactose induction increased the

level of Hsp104p relative to control cells grown in galactose (Menees and Sandmeyer, unpublished data). Ubiquitin-removing proteases can redirect proteins from degradative pathways in the cell (BAKER et al. 1992). In order to test whether Ty3 in *ssa1 ssa2* cells is targeted for degradation by ubiquitination, the effect of overexpressing *UBP3*, the gene for a ubiquitin-removing protease (a gift from E. Craig, University of Wisconsin), on Ty3 particle formation was determined. Expression of *UBP3* elevated levels of Ty3 Gag3 and CA, and promoted reverse transcription in the *ssa1 ssa2* mutants expressing Ty3. These observations showed that Ty3 particles are both an inducer and a substrate for the stress response system. In addition, these experiments suggest that retrotransposons provide access to genetic systems with which to study the positive and negative roles of chaperones and ubiquitin in retroviruslike replication.

6 Application of Particles Based on Heterologous Ty Fusions

The facility with which high levels of Ty proteins can be expressed with the concomitant formation of stable intracellular particles has had several novel applications. As early as 1987 ADAMS et al (1987b) and CLARE et al. (1988) showed that the reading frames of Ty1 could be fused to heterologous coding sequences and stable fusion proteins expressed. Adams et al. showed that when these fusion proteins included the Ty1 capsid domain, particles resulted which were effective immunogens. The utility of these polyvalent particles in eliciting cellular and humoral immune responses has been reviewed recently (ADAMS et al. 1995). In addition, fusion of proteins to Ty1 capsid have been used ingeniously to enhance detection and perhaps activity of reverse transcriptases including those of the LINE-like element CRE1 from the trypanosomatid *Crithidia fasciculata* (GABRIEL and BOEKE 1991), the human LINE element L1.2A (MATHIAS et al. 1991), the *coxI* intron i1 from the fungus *Podospora anserina* (FASSBENDER et al. 1994), and duck hepatitis virus B (TAVIS et al. 1994). In each case, conventional techniques had failed to demonstrate activity. It is clear that expression in yeast allowed high levels of protein to be produced and the multimerization mediated by Ty1 capsid facilitated concentration and purification of the protein for testing. Fortuitous features of this system are that substrates can diffuse readily into the particles and the proteins are relatively stable and therefore may be protected from proteolysis. It may also be that this context facilitates folding and multimerization by concentrating proteins within the cell. Whether these advantages are greater for RT, which naturally occurs in this context, or whether they could also apply to other proteins, the enzymatic assay of which has been problematic, remains to be tested. That non-RT species can be active within particles is suggested by the activity of the nuclease barnase in the Ty1 particle context

(NATSOUIS and BOEKE 1991). Fusion of barnase to Ty1 capsid was shown to have a dominant negative effect on activity of a Ty1 element expressed in *trans*. The long-range application of targeting heterologous enzymatic activities to retroviral particles is clear. These experiments have led directly to the demonstration of model retroviral capsid-nuclease fusions as antivirals (NATSOUIS et al. 1995).

7 Prospects

Genomic sequencing has only recently begun to suggest the extent of the representation of LTR retrotransposons in eukaryotic organisms. The study of these elements is in its infancy compared to investigations of retroviruses. Information about LTR retrotransposons has already posed interesting questions about the structure-function relationships of some retroviral proteins. In addition, it may provide insights into the evolution of retroviral mechanisms of replication which complement findings in retroviral systems. The coexistence of retrotransposons and retroviruses within single organisms like fruit flies and fish and the close relatedness of the gypsylike elements and viruses implies a potentially more direct interaction between these evolving types of retroelements than previously appreciated. Finally, the molecular characterization of particle morphogenesis of some of the elements described here will facilitate the application of genetics in fruit flies and yeast to the problem of the role of host proteins in particle morphogenesis.

In addition to common objectives with retrovirology, retrotransposon biology has separate objectives. The widespread nature of retrotransposons suggests that they may have significant cellular and genomic impact independent of their relationship to retroviruses, for example, the contribution of reverse transcriptase at different times in cellular development, the latent sculpting of target genomes by elements with insertion specificity, and the production of intracellular particles which may affect maintenance of the host cell stress response. Retrotransposons as naturally minimalist systems may have particular utility for applications, such as the design of retrovirus vectors with specificity or antiviral properties, the generation of effective antiviral immune responsiveness, or the study of catalytic proteins from pathogenic organisms. These developing avenues of investigation will continue to require investigation of the central problem of morphogenesis of the VLP structure.

References

- Adams SE, Mellor J, Gull K, Sim RB, Tuite MF, Kingsman SM, Kingsman AJ (1987a) The functions and relationships of Ty-VLP proteins in yeast reflect those of mammalian retroviral proteins. *Cell* 49: 111-119
- Adams SE, Dawson KM, Gull K, Kingsman SM, Kingsman AJ (1987b) The expression of hybrid HIV: Ty virus-like particles in yeast. *Nature* 329: 68-70
- Adams SE, Burns NR, Layton GT, Kingsman AJ (1995) Hybrid Ty virus-like particles. *Int Rev Immunol* 11: 133-141
- Arkhipova IR, Mazo AM, Cherkasova VA, Gorelova TV, Schuppe NG, Ilyin YV (1986) The steps of reverse transcription of *Drosophila* mobile dispersed genetic elements and U3-R-U5 structure of their LTRs. *Cell* 44: 555-563
- Atwood A, Lin J-H, Levin HL (1996) The retrotransposon Tf1 assembles virus-like particles that contain excess Gag relative to integrase because of a regulated degradation process. *Mol cell Biol* 16: 338-346
- Baker RT, Tobias JW, Varshavsky A (1992) Ubiquitin-specific proteases of *Saccharomyces cerevisiae*. *J Biol Chem* 267: 23364-23375
- Barat C, Lullien V, Schatz O, Keith G, Nugeyre MT, Gruninger-Leitch F, Barre-Sinoussi F, LeGrice SF, Darlix JL (1989) HIV-1 reverse transcriptase specifically interacts with the anticodon domain of its cognate primer tRNA. *EMBO J* 8: 3279-3285
- Belcourt MF, Farabaugh PJ (1990) Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. *Cell* 62: 339-352
- Bilanchone VW, Claypool JA, Kinsey PT, Sandmeyer SB (1993) Positive and negative regulatory elements control expression of the yeast retrotransposon Ty3. *Genetics* 134: 685-700
- Bingham PM, Zachar Z (1989) Retrotransposons and the FB transposon from *Drosophila melanogaster*. In: Berg DE, Howe MM (eds) *Mobile DNA*. American Society for Microbiology, Washington DC, pp 485-502
- Boeke JD, Corces VG (1989) Transcription and reverse transcription in retrotransposons. *Annu Rev Microbiol* 43: 402-433
- Boeke JD, Sandmeyer SB (1991) Yeast transposable elements. In: Broach JR, Jones EW, Pringle J (eds) *The molecular and cellular biology of the yeast Saccharomyces cerevisiae*. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 193-261
- Boeke JD, Garfinkel DJ, Styles CA, Fink GR (1985) Ty elements transpose through an RNA intermediate. *Cell* 40: 491-500
- Brierley C, Flavell AJ (1990) The retrotransposon copia controls the relative levels of its gene products post-transcriptionally by differential expression from its two major mRNAs. *Nucleic Acid Res* 18: 2947-2951
- Britten RJ (1995) Active *gypsy*/Ty3 retrotransposons or retroviruses in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 92: 599-601
- Brookman JL, Stott AJ, Cheeseman PJ, Burns NR, Adams SE, Kingsman AJ, Gull K (1995) An immunological analysis of Ty1 virus-like particle structure. *Virology* 207: 59-67
- Burns NR, Saibil HR, White NS, Pardon JF, Timmins PA, Mark S, Richardson H, Richards BM, Adams SE, Kingsman SM et al (1992) Symmetry, flexibility and permeability in the structure of yeast retrotransposon virus-like particles. *EMBO J* 11: 1155-1164
- Chapman KB, Byström AS, Boeke JD (1992) Initiator methionine tRNA is essential for Ty1 transposition in yeast. *Proc Natl Acad Sci USA* 89: 3236-3240
- Clare J, Farabaugh P (1985) Nucleotide sequence of a yeast Ty element: evidence for an unusual mechanism of gene expression. *Proc Natl Acad Sci USA* 82: 2829-2833
- Clare JJ, Belcourt M, Farabaugh PJ (1988) Efficient translational frameshifting occurs within a conserved sequence of the overlap between the two genes of a yeast Ty1 transposon. *Proc Natl Acad Sci USA* 85: 6816-6820
- Coffin JM (1990) *Retroviridae* and their replication. In: Fields BN, Knipe DM (eds) *Virology*, 2nd edn. Raven, New York, pp 1437-1500
- Craig EA, Jacobsen K (1985) Mutations in cognate genes of *Saccharomyces cerevisiae* *hsp 70* result in reduced growth rates at low temperatures. *Mol Cell Biol* 5: 3517-3524
- Craig EA, Gambill BD, Nelson RJ (1993) Heat shock proteins: molecular chaperones of protein biogenesis. *Microbiol Rev* 57: 402-414

- Curcio MJ, Garfinkel DJ (1991a) Regulation of retrotransposition in *Saccharomyces cerevisiae*. *Mol Microbiol* 5: 1823-1829
- Curcio MJ, Garfinkel DJ (1991b) Single-step selection for Ty1 element retrotransposition. *Proc Natl Acad Sci USA* 88: 936-940
- Curcio MJ, Garfinkel DJ (1992) Posttranslational control of Ty1 retrotransposition occurs at the level of protein processing. *Mol Cell Biol* 12: 2813-2825
- Curcio MJ, Garfinkel DJ (1994) Heterogeneous functional Ty1 elements are abundant in the *Saccharomyces cerevisiae* genome. *Genetics* 136: 1245-1259
- Curcio MJ, Sanders NJ, Garfinkel DJ (1988) Transpositional competence and transcription of endogenous Ty elements in *Saccharomyces cerevisiae*: implications for regulation of transposition. *Mol Cell Biol* 8: 3571-3581
- Dinman JD, Icho T, Wickner RB (1991) A -1 ribosomal frameshift in a double-stranded RNA virus of yeast forms a *gag-pol* fusion protein. *Proc Natl Acad Sci USA* 88: 174-178
- Eichinger DJ, Boeke JD (1988) The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. *Cell* 54: 955-966
- Eichinger DJ, Boeke JD (1990) A specific terminal structure is required for Ty1 transposition. *Genes Dev* 4: 324-330
- Emori Y, Shiba S, Inouye I, Yuki S, Saigo K (1985) The nucleotide sequences of copia and copia-related RNA in *Drosophila* virus-like particles. *Nature* 315: 773-776
- Evgen'ev MB, Corces VG, Lankenau D-H (1992) Ulysses transposable element of *Drosophila* shows high structural similarities to functional domains of retroviruses. *J Mol Biol* 225: 917-924
- Falkenthal S, Lengyel JA (1980) Structure, translation, and metabolism of the cytoplasmic copia ribonucleic acid of *Drosophila melanogaster*. *Biochemistry* 19: 5842-5850
- Farabaugh PJ, Zhao H, Pande S, Vimaladithan A (1993) Translational frameshifting expresses the *POL3* gene of retrotransposon Ty3 of yeast. *Cell* 74: 93-103
- Fassbender S, Bruhl K-H, Ciriacy M, Kuck U (1994) Reverse transcriptase activity of an intron encoded polypeptide. *EMBO J* 13: 2075-2083
- Fields S, Song O (1989) A novel genetic system to detect protein-protein interactions. *Nature* 340: 245-246
- Finnegan DJ (1989) F and related elements in *Drosophila melanogaster*. In: Berg DE, Howe MM (eds) *Mobile DNA*. American Society for Microbiology, Washington DC, pp 519-529
- Flavell AJ (1984) Role of reverse transcription in the generation of extrachromosomal copia mobile genetic elements. *Nature* 310: 514-516
- Flavell AJ (1992) Ty1-copia group retrotransposons and the evolution of retroelements in the eukaryotes. *Genetica* 86: 203-214
- Flavell AJ, Ish-Horowicz D (1981) Extrachromosomal circular copies of the eukaryotic transposable element copia in cultured *Drosophila* cells. *Nature* 292: 591-595
- Flavell AJ, Ish-Horowicz D (1983) The origin of extrachromosomal circular copia elements. *Cell* 34: 415-419
- Flavell AJ, Smith DB (1992) A Ty1-copia group retrotransposon sequence in a vertebrate. *Mol Gen Genet* 233: 322-326
- Flavell AJ, Ruby SW, Toole JJ, Roberts BE, Rubin GM (1980) Translation and developmental regulation of RNA encoded by the eukaryotic transposable element copia. *Proc Natl Acad Sci USA* 77: 7107-7111
- Flavell AJ, Dunbar E, Anderson R, Pearce SR, Hartly R, Kumar A (1995) Ty1-copia group retrotransposons are ubiquitous and heterogeneous in higher plants. *Nucleic Acids Res* 23: 3639-3644
- Friesen PD, Nissen MS (1990) Gene organization and transcription of TED, a lepidopteran retrotransposon integrated within the baculovirus genome. *Mol Cell Biol* 10: 3067-3077
- Fuettner J, Hohn T (1987) Involvement of nucleocapsids in reverse transcription: a general phenomenon? *Trends Biochem Sci* 12: 92-95
- Fulton AM, Mellor J, Dobson MJ, Chester J, Warmington JR, Indge KJ, Oliver SG, de la PP, Wilson W, Kingsman AJ et al (1985) Variants within the yeast Ty sequence family encode a class of structurally conserved proteins. *Nucleic Acids Res* 13: 4097-4112
- Furuichi T, Inouye S, Inouye M (1987) Biosynthesis and structure of stable branched RNA covalently linked to the 5' end of multicopy single-stranded DNA of *Stigmatella auranticaca*. *Cell* 48: 55-62
- Gabriel A, Boeke JD (1991) Reverse transcriptase encoded by a retrotransposon from the trypanosomatid *Crithidia fasciculata*. *Proc Natl Acad Sci USA* 88: 9794-9798

- Garfinkel DJ, Boeke JD, Fink GR (1985) Ty element transposition: reverse transcriptase and virus-like particles. *Cell* 42: 507-517
- Garfinkel DJ, Hedge AM, Youngren SD, Copeland TD (1991) Proteolytic processing of pol-TYB proteins from the yeast retrotransposon Ty1. *J Virol* 65: 4573-4581
- Grandbastien M-A, Spielmann A, Caboche M (1989) Tnt1, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. *Nature* 337: 376-380
- Hansen LJ, Sandmeyer SB (1990) Characterization of a transpositionally active Ty3 element and identification of the Ty3 integrase protein. *J Virol* 64: 2599-2607
- Hansen LJ, Chalker DL, Sandmeyer SB (1988) Ty3, a yeast retrotransposon associated with tRNA genes, has homology to animal retroviruses. *Mol Cell Biol* 8: 5245-5256
- Hansen LJ, Chalker DL, Orlinsky KJ, Sandmeyer SB (1992) Ty3 GAG3 and POL3 genes encode the components of intracellular particles. *J Virol* 66: 1414-1424
- Hauber J, Nelböck-Hochstetter P, Feldmann H (1985) Nucleotide sequence and characteristics of a Ty element from yeast. *Nucleic Acids Res* 13: 2745-2758
- Hu JC, Dahlberg JE (1983) Structural features required for the binding of tRNA^{Trp} to avian myeloblastosis virus reverse transcriptase. *Nucleic Acids Res* 11: 4823-4833
- Inouye S, Yuki S, Saigo K (1986) Complete nucleotide sequence and genome organization of a *Drosophila* transposable genetic element, 297. *Eur J Biochem* 154: 417-425
- Jacks T (1989) Translational suppression in gene expression in retroviruses and retrotransposons. In: Swanstrom R, Vogt PK (eds) *Retroviruses. Strategies of replication*. Springer, Berlin Heidelberg New York, pp 93-124 (Current topics in microbiology and immunology, vol 157)
- Jacks T, Townsley K, Varmus HE, Majors J (1987) Two efficient ribosomal frameshifting events are required for synthesis of mouse mammary tumor virus gag-related polyproteins. *Proc Natl Acad Sci USA* 84: 4298-4302
- Jacks T, Power MD, Masiaz FR, Luciw PA, Barr PJ, Varmus HE (1988) Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. *Nature* 331: 280-283
- Janetzky B, Lehle L (1992) Ty4, a new retrotransposon from *Saccharomyces cerevisiae*, flanked by tau-elements. *J Biol Chem* 267: 19798-19805
- Kawakami K, Pande S, Faiola B, Moore DP, Boeke JD, Farabaugh PJ, Strathern JN, Nakamura Y, Garfinkel DJ (1993) A rare tRNA-Arg(CCU) that regulates Ty1 element ribosomal frameshifting is essential for Ty1 retrotransposition in *Saccharomyces cerevisiae*. *Genetics* 135: 309-320
- Keeney JB, Chapman KB, Lauerma V, Voytas DF, Åström SU, von Pawel-Rammingen U, Byström A, Boeke JD (1995) Multiple molecular determinants for retrotransposition in a primer tRNA. *Mol Cell Biol* 15: 217-226
- Kikuchi Y, Ando Y, Shiba T (1986) Unusual priming mechanism of RNA-directed DNA synthesis in copia retrovirus-like particles of *Drosophila*. *Nature* 323: 824-826
- Kim A, Terzian C, Santamaria P, Pélissier A, Prud'homme N, Bucheton A (1994) Retroviruses in invertebrates: the gypsy retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 91: 1285-1289
- Kinsey P, Sandmeyer S (1995) Ty3 transposes in mating populations of yeast: a novel transposition assay for Ty3. *Genetics* 139: 81-94
- Kirchner J, Sandmeyer S (1993) Proteolytic processing of Ty3 proteins is required for transposition. *J Virol* 67: 19-28
- Kirchner J, Sandmeyer SB (1996) Ty3 integrase cleaves a dinucleotide from the 3' termini of Ty3 DNA and may have a role in reverse transcription: analysis of integrase mutations. *J Virol* (in press)
- Kirchner J, Sandmeyer SB, Forrest DB (1992) Transposition of Ty3 GAG3-POL3 fusion mutant is limited by availability of capsid protein. *J Virol* 66: 6081-6092
- Lankenau DH, Huijser P, Jansen E, Miedema K, Hennig W (1988) Micropia: a retrotransposon of *Drosophila* combining structural features of DNA viruses, retroviruses and non-viral transposable elements. *J Mol Biol* 204: 233-246
- Lauerma V, Boeke JD (1994) The primer tRNA sequence is not inherited during Ty1 transposition. *Proc Natl Acad Sci USA* 91: 9847-9851
- Leis J, Baltimore D, Bishop JM, Coffin J, Fleissner E, Goff SP, Oroszlan S, Robinson H, Skalka AM, Temin HM et al. (1988) Standardized and simplified nomenclature for proteins common to all retroviruses. *J Virol* 62: 1808-1809
- Levin HL (1995) A novel mechanism of self-primed reverse transcription defines a new family of retroelements. *Mol Cell Biol* 15: 3310-3317
- Levin HL, Boeke JD (1992) Demonstration of retrotransposition of the Tf1 element in fission yeast. *EMBO J* 11: 1145-1153

- Levin HL, Weaver DC, Boeke JD (1990) Two related families of retrotransposons from *Schizosaccharomyces pombe*. *Mol Cell Biol* 10: 6791-6798
- Levin HL, Weaver DC, Boeke JD (1993) Novel gene expression mechanism in a fission yeast retroelement: Tf1 proteins are derived from a single primary translation product. *EMBO J* 12: 4885-4895
- Mammano F, Hagen Å, Hoglund S, Göttinger HG (1994) Role of the major homology region of human immunodeficiency virus type 1 in virion morphogenesis. *J Virol* 68: 4927-4936
- Marlor RL, Parkhurst SM, Corces VG (1986) The *Drosophila melanogaster* gypsy transposable element encodes putative gene products homologous to retroviral proteins. *Mol Cell Biol* 6: 1129-1134
- Mathias SL, Scott AF, Kazanian Jr, HH, Boeke JD, Gabriel A (1991) Reverse transcriptase encoded by a human transposable element. *Science* 254: 1808-1810
- McHale MT, Roberts IN, Noble SM, Beaumont C, Whitehead MP, Seth D, Oliver RP (1992) CFT-1: an LTR-retrotransposon in *Cladosporium fulvum*, a fungal pathogen of tomato. *Mol Gen Genet* 233: 337-347
- Mellor J, Fulton AM, Dobson MJ, Roberts NA, Wilson W, Kingsman AJ, Kingsman SM (1985a) The Ty transposon of *Saccharomyces cerevisiae* determines the synthesis of at least three proteins. *Nucleic Acids Res* 13: 6249-6263
- Mellor J, Malim MH, Gull K, Tuite MF, McCready S, Dibbayawan T, Kingsman SM, Kingsman AJ (1985b) Reverse transcriptase activity and Ty RNA are associated with virus-like particles in yeast. *Nature* 318: 583-586
- Menees TM, Sandmeyer SB (1994) Transposition of the yeast retroviruslike element Ty3 is dependent on the cell cycle. *Mol Cell Biol* 14: 8229-8240
- Menees TM, Sandmeyer SB (1996) Ubiquitination and inhibition of yeast retroviruslike element Ty3 particle formation. *Proc Natl Acad Sci USA* (in press)
- Miller K, Rosenbaum J, Zbrzezna V, Pogo AO (1995) The nucleotide sequence of *Drosophila melanogaster* copia-specific 2.1 kb mRNA. *Nucleic Acids Res* 17: 2134
- Miyake T, Mae N, Shiba T, Kondo S (1987) Production of virus-like particles by the transposable genetic element, copia, of *Drosophila melanogaster*. *Mol Gen Genet* 207: 29-37
- Mount SM, Rubin GM (1985) Complete nucleotide sequence of the *Drosophila* transposable element copia: homology between copia and retroviral proteins. *Mol Cell Biol* 5: 1630-1638
- Müller F, Bruhl KH, Freidel K, Kowalik KV, Ciriacy M (1987) Processing of Ty1 proteins and formation of Ty1 virus-like particles in *Saccharomyces cerevisiae*. *Mol Gen Genet* 207: 421-429
- Müller F, Laufer W, Pott U, Ciriacy M (1991) Characterization of products of Ty1-mediated reverse transcription in *Saccharomyces cerevisiae*. *Mol Gen Genet* 226: 145-153
- Natsoulis G, Boeke JD (1991) New antiviral strategy using capsid-nuclease fusion proteins. *Nature* 352: 632-635
- Natsoulis G, Seshiah P, Federspiel MJ, Rein A, Hughes SH, Boeke JD (1995) Targeting of a nuclease to murine leukemia virus capsids inhibits viral multiplication. *Proc Natl Acad Sci USA* 92: 364-368
- Oliver SG et al (1992) The complete DNA sequence of yeast chromosome III. *Nature* 357: 38-46
- Orlinsky KJ, Sandmeyer SB (1994) The Cys-His motif of Ty3 NC can be contributed by Gag3 or Gag3-Pol3 polyproteins. *J Virol* 68: 4152-4166
- Orlinsky KJ, Gu J, Hoyt M, Sandmeyer SB, Menees TM (1996) Mutations in the Ty3 major homology region affect multiple steps in Ty3 retrotransposition. *J Virol* (in press)
- Paquin CE, Williamson VM (1988) Effect of temperature on Ty transposition. *Ban Rpt* 30: 235-243
- Parsell DA, Lindquist S (1993) The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu Rev Genet* 27: 437-496
- Parsell DA, Kowal AS, Singer MA, Lindquist S (1994) Protein disaggregation mediated by heat-shock protein Hsp 104. *Nature* 372: 475-478
- Péllissier A, Song SU, Prud'homme N, Smith PA, Bucheton A, Corces VG (1994) Gypsy transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the *Drosophila flamenco* gene. *EMBO J* 13: 4401-4411
- Plesset J, Palm C, McLaughlin CS (1982) Induction of heat shock proteins and thermotolerance by ethanol in *Saccharomyces cerevisiae*. *Bioc Biophys Res Comm* 108: 1340-1345
- Pochart P, Agoutin B, Rousset S, Chanet R, Doroszkiewicz V, Heyman T (1993a) Biochemical and electron microscope analyses of the DNA reverse transcripts present in the virus-like particles of the yeast transposon Ty1. Identification of a second origin of Ty1 DNA plus strand synthesis. *Nucleic Acids Res* 21: 3513-3520

- Pochart P, Agoutin B, Fix C, Keith G, Heyman T (1993b) A very poorly expressed tRNA^{Ser} is highly concentrated together with replication primer initiator tRNA^{Met} in the yeast Ty1 virus-like particles. *Nucleic Acids Res* 21: 1517-1521
- Saigo K, Kugimiya W, Matsuo Y, Inouye S, Yoshioka K, Yuki S (1984) Identification of the coding sequence for a reverse transcriptase-like enzyme in a transposable genetic element in *Drosophila melanogaster*. *Nature* 312: 659-661
- Scheinker VS, Lozovskaya ER, Bishop JG, Corces VG, Evgen'ev MB (1990) A long terminal repeat-containing retrotransposon is mobilized during hybrid dysgenesis in *Drosophila virilis*. *Proc Natl Acad Sci USA* 87: 9615-9619
- Sharon G, Burkett TJ, Garfinkel DJ, (1994) Efficient homologous recombination of Ty1 element cDNA when integration is blocked. *Mol Cell Biol* 14: 6540-6541
- Shiba T, Saigo K (1983) Retrovirus-like particles containing RNA homologous to the transposable element copia in *Drosophila melanogaster*. *Nature* 302: 119-124
- Smyth DR, Kalitsis P, Joseph JL, SENTRY JW (1989) Plant retrotransposon from *Lilium henryi* is related to Ty3 of yeast and the gypsy group of *Drosophila*. *Proc Natl Acad Sci USA* 86: 5015-5019
- Song Su, Gerasimova T, Kurkulos M, Boeke JD, Corces V (1994) An Env-like protein encoded by a *Drosophila* retroelement: evidence that gypsy is an infectious retrovirus. *Genes Dev* 8: 2046-2057
- Springer MS, Britten RJ (1993) Phylogenetic relationships of reverse transcriptase and RNase H sequences and aspects of genome structure in the gypsy group of retrotransposons. *Mol Biol Evol* 10: 1370-1379
- Springer MS, Davidson EH, Britten RJ (1991) Retroviral-like element in a marine invertebrate. *Proc Natl Acad Sci USA* 88: 8401-8404
- Stone DE, Craig EA (1990) Self-regulation of 70-kilodalton heat shock proteins in *Saccharomyces cerevisiae*. *Mol Cell Biol* 10: 1622-1632
- Strambio-de-Castillia C, Hunter E (1992) Mutational analysis of the major homology region of Mason-Pfizer monkey virus by use of saturation mutagenesis. *J Virol* 66: 7021-7032
- Stucka R, Lochmüller H, Feldmann H (1989) Ty4, a novel low-copy number element in *Saccharomyces cerevisiae*: one copy is located in a cluster of Ty elements and tRNA genes. *Nucleic Acids Res* 17: 4993-5001
- Syomin BV, Kandror KV, Semakin AB, Tsuprun VL, Stepanov AS (1993) Presence of the gypsy (MDG4) retrotransposon in extracellular virus-like particles. *FEBS Lett* 323: 285-288
- Tanda S, Shrimpton AE, Chueh LL, Itayama H, Matsubayashi H, Saigo K, Tobari YN, Langley CH (1988) Retrovirus-like features and site specific insertions of a transposable element, tom, in *Drosophila ananassae*. *Mol Gen Genet* 214: 405-411
- Tanda S, Mullor J, Corces VG (1994) The *Drosophila* tom retrotransposon encodes an envelope protein. *Mol Cell Biol* 14: 5392-5401
- Tavis JE, Perri S, Ganem D (1994) Hepadnavirus reverse transcription initiates within the stem-loop of the RNA packaging signal and employs a novel strand transfer. *J Virol* 68: 3536-3543
- Tchenio T, Heidmann T (1991) Defective retroviruses can disperse in the human genome by intracellular transposition. *J Virol* 65: 2113-2118
- Telesnitsky A, Goff SP (1993) Strong-stop strand transfer during reverse transcription. In: Skalka AM, Goff SP (eds) *Reverse transcriptase*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 49-83
- Vimaladithan A, Farabaugh PJ (1994) Special peptidyl-tRNA molecules can promote transitional frameshifting without slippage. *Mol Cell Biol* 14: 8107-8116
- Voytas DF, Ausubel FM (1988) A copia-like transposable element family in *Arabidopsis thaliana*. *Nature* 336: 242-244
- Voytas DF, Boeke JD (1992) Yeast retrotransposon revealed. *Nature* 358: 717
- Voytas DF, Boeke JD (1993) Yeast retrotransposons and tRNAs. *TIGS* 9: 421-427
- Warmington JR, Waring RB, Newlon CS, Indge KJ, Oliver SG (1985) Nucleotide sequence characterization of Ty1-17, a class II transposon from yeast. *Nucleic Acids Res* 13: 6679-6693
- Wills JW, Craven RC (1991) Form, function, and use of retroviral gag proteins (editorial). *AIDS* 5: 639-654
- Xiong Y, Eickbush TH (1990) Origin and evolution of retroelements based upon their reverse transcriptase sequences. *EMBO J* 9: 3353-3362
- Xu H, Boeke JD (1990a) Host genes that influence transposition in yeast: the abundance of a rare tRNA regulates Ty1 transposition frequency. *Proc Natl Acad Sci USA* 87: 8360-8364
- Xu H, Boeke JD (1990b) Localization of sequences required in cis for yeast Ty1 element transposition near the long terminal repeats: analysis of mini-Ty1 elements. *Mol Cell Biol* 10: 2695-2702

- Xu H, Boeke JD (1991) Inhibition of Ty1 transposition by mating pheromones in *Saccharomyces cerevisiae*. *Mol Cell Biol* 11: 2736-2743
- Yoshioka K, Honma H, Zushi M, Kondo S, Togashi S, Miyake T, Shiba T (1990) Virus-like particle formation of *Drosophila copia* through autocatalytic processing. *EMBO J* 9: 535-541
- Yoshioka K, Fujita A, Kondo S, Miyake T, Sakaki Y, Shiba T (1992) Production of a unique multi-lamella structure in the nuclei of yeast expressing *Drosophila copia gag* precursor. *FEBS Lett* 302: 5-7
- Youngren SD, Boeke JD, Sanders NJ, Garfinkel DJ (1988) Functional organization of the retrotransposon Ty from *Saccharomyces cerevisiae*: Ty protease is required for transposition. *Mol Cell Biol* 8: 1421-1431
- Yuki S, Inouye S, Ishimaru S, Saigo K (1986) Nucleotide sequence characterization of a *Drosophila* retrotransposon, 412. *Eur J Biochem* 158: 403-410
- Zou S, Wright DA, Voytas DF (1995) The *Saccharomyces* Ty5 retrotransposon family is associated with origins of DNA replication at the telomeres and the silent mating locus *HMR*. *Proc Natl Acad Sci USA* 92: 920-924
- Zou S, Ke N, Kim JM, Voytas DF (1996) The *Saccharomyces* retrotransposon Ty5 integrates preferentially into regions of silent chromatin at the telomeres and mating loci. *Genes Dev* 10: 634-645