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Two Related Families of Retrotransposons from *Schizosaccharomyces pombe*

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Two related families of transposons were isolated from *Schizosaccharomyces pombe*, an organism which has been the object of extensive genetic studies which had previously produced no evidence for the existence of such elements. These two classes of repeated DNAs, dubbed Tf1 (transposon of fission yeast 1) and Tf2 have many properties of retrotransposons. Tf1 and Tf2 both possess long terminal repeats and predicted protein sequences that resemble the protease, reverse transcriptase, and integrase domains of retroviruses. The chromosomal locations and total numbers of Tf1 and Tf2 differ greatly in various isolates of *S. pombe*. The Tf elements are expressed in the form of 4.5-kb mRNAs. The complete sequence of Tf1 was determined and suggests that a novel mechanism for regulating its gene expression may be used.

Transposable elements are thought to be present in all cell types, yet no such element has previously been reported from *Schizosaccharomyces pombe*. Transposable elements isolated from *Saccharomyces cerevisiae* have been characterized to develop an understanding of the molecular mechanisms of transposition in eucaryotic cells (3, 4, 10). We examined the genome of the related yeast *S. pombe* for transposable elements, since some of its biological properties, including chromosome condensation and centromere structure, have been shown to be more similar to those of higher eucaryotic cells than to those of *S. cerevisiae* (15, 40). We initially searched unsuccessfully for transposons in the well-known strains *S. pombe* 972 and 975 studies by Leupold et al. (14) by using a genetic approach similar to those used successfully with brewer's yeast, *S. cerevisiae*, to identify transposition events (9, 29, 37). We then initiated a study of repetitive DNAs isolated from *S. pombe* strains in other laboratories. As a result, we isolated two families of retrotransposons, transposon of fission yeast 1 (Tf1) and Tf2. The sequence of Tf1 was determined and found to contain an open reading frame of 1,340 amino acids that has regions of sequence identity to the protease (PR), reverse transcriptase (RT), and integrase (IN) domains of retroviruses. In addition, RNA blot analysis demonstrated that both Tf1 and Tf2 produce 4.5-kb transcripts that are of sufficient size to be full-length copies.

Isolation and characterization of Tf element clones. The *S. pombe* strains used in this study and the element copies cloned from them are described in Table 1. The Tf element clones were identified as follows. We found that probes made from the pEE102 plasmid insert that hybridized to multiple *S. pombe* DNA sequences mapped within a 1.0-kbp *Dra*I fragment. (This fragment was later shown to contain a single Tf1 long terminal repeat [LTR]). This probe hybridized intensely to a 1.6-kbp *Sna*BI fragment in genomic DNAs from a variety of *S. pombe* strains (data not shown). Such a fragment was cloned directly by eluting appropriate-size DNA from an agarose gel on which *Sna*BI-digested strain 972 DNA had been run and ligating it into *Sma*I-digested M13mp8, giving rise to clone M13-*Sna*BI-6. Restriction mapping and limited sequencing enabled us to identify an internal region of the retrotransposon which extended from the internal *Xmn*I site at position 4099 to an *Ssp*I site at position 4733. This probe was used to isolate Tf1-107 from a genomic library of *S. pombe* NCYC 132. Sections of Tf1-107 were ligated into M13mp18 and M13mp19 and sequenced by the *Exo*III nuclease S1 deletion (17) and dideoxy (35) methods. The sequences of the other LTRs were obtained by sequencing of plasmids which had lost the internal Tf sequences by in vivo LTR-LTR recombination events. These plasmids were obtained by transforming *Escherichia coli* with plasmids which had been linearized within the internal Tf1 sequence and screening for plasmids lacking the internal segment of Tf1 by restriction analysis. The plasmid DNA was isolated from 1.5-ml cultures of saturated DH5α cells (19); after isopropanol precipitation, the DNA was suspended in 40 μl of TE (10 mM Tris hydrochloride, pH 7.7, 1 mM EDTA). One-quarter (10 μl) of each DNA preparation was denatured by adding 6 μl of H_2O and 4 μl of a 1 M NaOH–1 mM EDTA solution. The denatured DNA was precipitated by addition of 4 μl of 10 M ammonium acetate and 100 μl of ethanol, followed by incubation for 20 min at −20°C. The pellets were suspended in 7 μl of H_2O and used directly in the sequencing reactions of the Sequenase kit (United States Biochemical Corp.).

Plasmid constructions. Plasmid pHL117-17 contains a copy of Tf1-107 transcribed by its own promoter and was constructed by ligating the origin-containing *Bgl*II-*Xho*I fragment of pCYH20 (18) to a *Bam*HI-*Xho*I fragment that contains the intact Tf1-107 element. Plasmid pHL190-2 contains a fusion of the *S. pombe* fructose bisphosphatase (*fbp*) promoter to Tf1-107. The *S. pombe* origin and *fbp* promoter were both supplied on a *Bam*HI-*Xho*I fragment from pCYH20 that was ligated to an *Xho*I-*Bam*HI fragment of Tf1-107 that extends from a site within the 5′ LTR through the element to a *Bam*HI site in the 3′-flanking DNA. The *fbp* promoter was fused to Tf1 at an *Xho*I site that had been previously introduced within the 5′ LTR at the transcription start site (details to be published elsewhere).

Genomic DNA blotting. Genomic DNA was isolated from *S. pombe* for DNA blot analysis by using a glass bead-phenol

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TABLE 1. Strains

Strain	Genotype	Origin	Transposon(s)	Source
972	h^-	Switzerland[a]	Solo LTR, Tf2-21, Tf2-22	J. Kohli
975	h^+	Switzerland[a]		J. Kohli
NCYC 132	Wild type	Unknown[a]	Tf1-107	H. Gutz
CSIR Y-457	Wild type	Swaziland[a]		H. Gutz
0207	Wild type	Germany[a]	Tf2-111	H. Gutz
0208	Wild type	Holland[a]	Tf1-112	H. Gutz
YHL232	*ura4-D18* h^-	Switzerland[b]		J. Kohli[c]
YHL351	YHL232(pHL117-17)	Switzerland[b]		This report
YHL410	YHL232(pHL190-2)	Switzerland[b]		This report
YHL211	*ade6-704 ura2-10 his7-366 ade2-17 lys7-1 h*	Switzerland[b]		This report[d]
123-20	*ade6-704 ura2-10 his7-366* h^-	Switzerland[b]		J. Kohli
13-81	*ade6-704 ura2-10 lys7-1 ade2-17* h^+	Switzerland[b]		J. Kohli

[a] The references for these strains are listed in reference 43.
[b] These strains are all derivatives of Leupold strains 972 and 975.
[c] Reference 13.
[d] YHL211 was derived from the cross 123-20 (h^-) × 13-81 (h^+).

protocol. A 10-ml volume of a saturated *S. pombe* culture was pelleted and suspended in 0.2 ml of extraction buffer (0.5 M NaCl, 0.2 M Tris chloride [pH 7.6], 0.01 M EDTA, 1.0% sodium dodecyl sulfate). After the cells were transferred to an Eppendorf tube, 0.4 g of glass beads was added (before use, the 0.45- to 0.5-mm-diameter beads were washed by soaking in concentrated HNO_3 overnight, washed extensively with distilled H_2O, and baked dry). A 0.2-ml volume of PCIA (25 ml of phenol, 24 ml of chloroform, 1 ml of isoamyl alcohol, all equilibrated with an equal volume of the extraction buffer minus sodium dodecyl sulfate) was added, and the tube was vortexed at top speed for 2.5 min. A 0.3-ml volume of extraction buffer and 0.3 ml of PCIA were added, and the tube was vortexed for 1.0 min. The sample was spun for 5.0 minutes in an Eppendorf centrifuge, and the aqueous phase was reextracted with an equal volume of PCIA. The extractions were continued until the interphases were clean. Two volumes of ethanol was added to precipitate the DNA. The pellets were washed in 70% ethanol and suspended in 50 μl of TE plus 1 μl of a 2-mg/ml solution of boiled RNase A. Five-microliter volumes of the preparations were restriction digested in 20-μl reaction mixtures and loaded onto agarose gels for DNA blotting as previously described (24).

RNA isolation and blotting. RNA was isolated from *S. pombe* by the same procedure, except that the samples were chilled at all times and after ethanol precipitation, the RNA was suspended in 100 μl of loading dye (50% formamide, 25% H_2O, 5.5% formaldehyde, 10% MOPS (morpholinepropanesulfonic acid) solution containing 0.2 M MOPS [pH 7.0], 0.05 M sodium acetate, 0.01 M EDTA). When indicated, RNA was poly(A)$^+$ selected as described by Maniatis et al. (24) and 20 μg of RNA was loaded into each lane of a 1.0% agarose gel that contained 10% MOPS solution and 6% formaldehyde. The RNA samples were transferred onto Hybond-N membrane as suggested by Amersham, the manufacturer.

Nucleotide sequence accession number. Sequence data (obtained in their entirety from both strands) for Tf1-107 are available under GenBank accession no. M38526.

RESULTS AND DISCUSSION

Isolation and structure of retrotransposon Tf1. Our study of repeated *S. pombe* DNAs isolated in other laboratories included a genomic clone, pEE102, kindly provided by T. Matsumoto and M. Yanagida (Kyoto University, Kyoto, Japan), that contains a sequence that is highly repeated in the *S. pombe* genome. By extracting other copies of this repeat from our *S. pombe* genomic DNA libraries and sequencing them, we found that the fragment from pEE102 contained a single copy of an LTR of intact retrotransposon Tf1. To isolate intact copies of the element, we constructed and screened four genomic DNA libraries and identified 15 full-length copies of the retrotransposons from four distinct strains of *S. pombe* (Table 1) by hybridization. Both strands of one such copy, Tf1-107, were sequenced and found to have many similarities to retroviruses that are often observed in retrotransposons. The DNA and protein sequences of Tf1-107 are shown in Fig. 1.

Tf1-107 has identical 358-base LTRs flanking the internal sequence in direct orientation. Each LTR begins with TG and ends with CA, as do LTRs from all retroviruses and most retrotransposons (2, 41). Another aspect of Tf1-107 that is similar to retroviruses and retrotransposons is the long (11 of 12 nucleotides) polypurine stretch located just 5′ of the 3′ LTR (Fig. 1) that may be necessary to prime plus-strand reverse transcription of the element.

The internal section of Tf1-107 contains one continuous open reading frame which potentially encodes a protein of 1,340 amino acids (Fig. 1). The amino acid sequence of the hypothetical protein contains regions similar to the PR, RT, RNase H, and IN domains of *pol* genes from both retroviruses and retrotransposons (8). An additional element of the Tf1 family, Tf1-112, was sequenced from a region 420 bases upstream of the start of the PR domain to the beginning of the RT domain. This entire region was found to be identical to the sequence of Tf1-107, indicating that this copy also lacks a frameshift between the putative *gag* and *pol* regions.

Although the C terminus of the predicted protein is similar to that of *pol* proteins, the N-terminal 237-amino-acid sequence is not similar to any published *gag* sequences. *gag* sequences, however, are among the less well-conserved coding sequences of retroviruses and retrotransposons (8). Nevertheless, the size and position of the 237 amino acids upstream of the identifiable *pol* domains suggest that the N-terminal domain possesses CA (capsid protein)-like functions. No homology to the putative nucleic acid-binding domain found in many *gag* proteins (7) was observed in Tf1. This is also true of retrotransposon Ty1, which is nevertheless known to direct synthesis of viruslike particles (12, 27).

A second *S. pombe* retrotransposon, Tf2. Comparison of

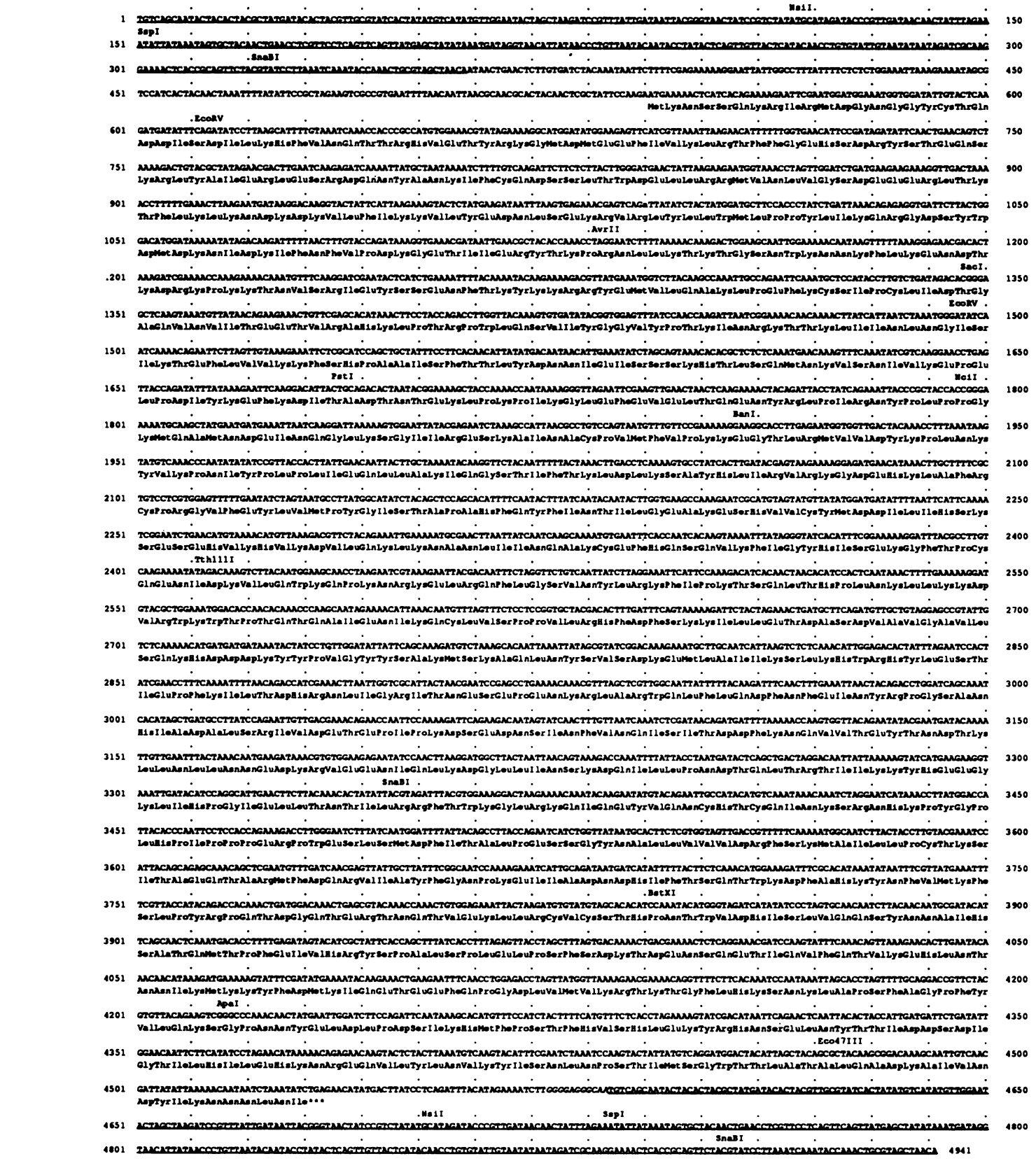

FIG. 1. Sequence of Tf1-107. The DNA sequences of both LTRs are underlined, and the polypurine stretch is in italics. The positions of various restriction sites are shown.

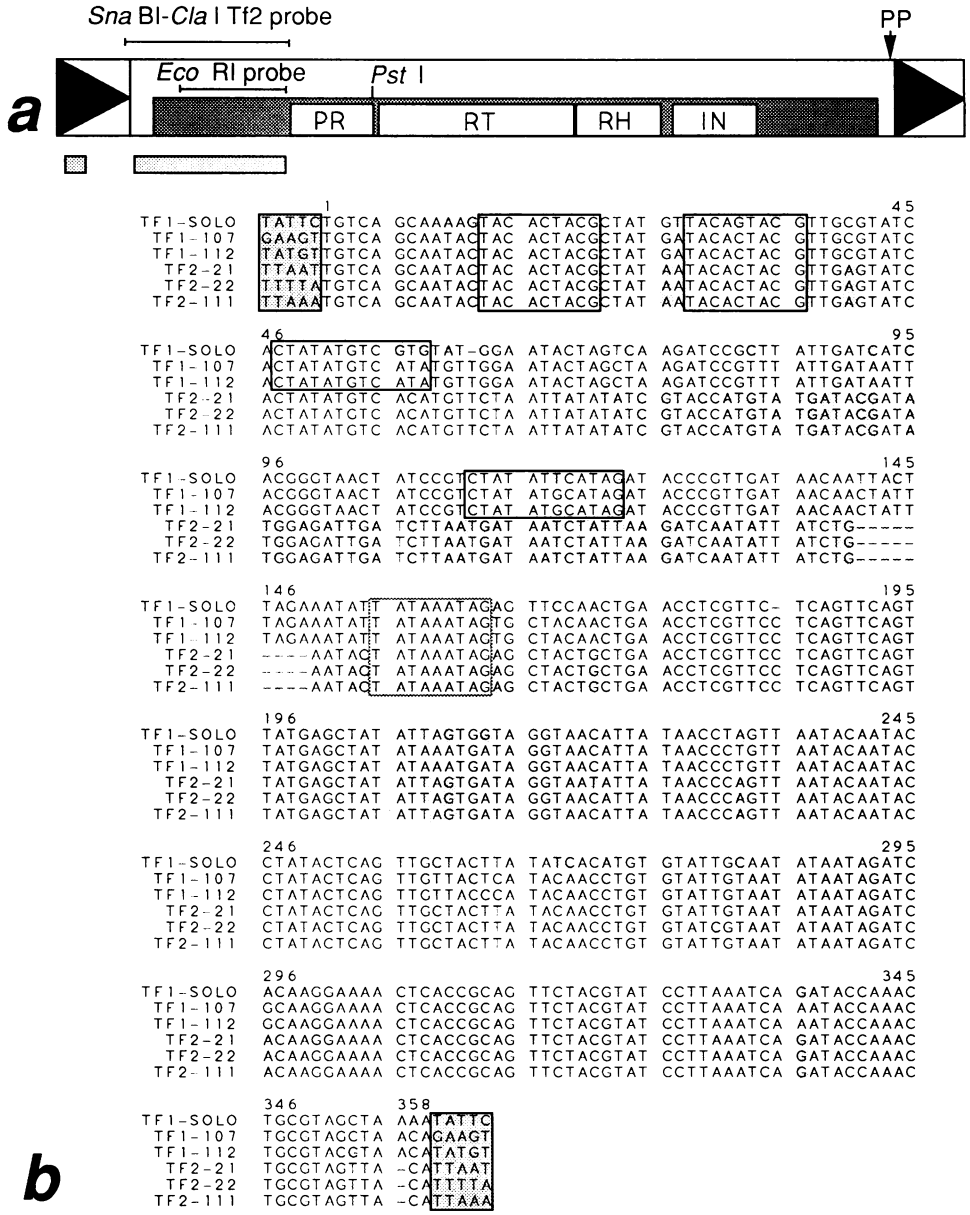

FIG. 2. Features of Tf elements. (a) Structural aspects of the Tf1-107 sequence. The shaded portion of the element represents the open reading frame, with functional domains positioned on the basis of amino acid similarity to the PR, RT, RNase H (RH), and IN domains of retroviruses (8). The triangles depict the 358-bp LTRs. Restriction sites referred to in the text are shown. The 12 nucleotides of Tf1 adjacent to the 3′ LTR are designated PP (polypurine). The shaded bars indicate the sections within Tf2 elements that are less than 75% identical to Tf1 as determined by DNA sequence analysis and Southern blotting. (b) DNA sequence alignment of six Tf element LTRs. The shaded residues are those that differ from the Tf1-107 sequence. The 5-base target site duplications are shaded and enclosed by solid-line boxes, the TATA boxes are enclosed in a wavy-line box, and both the 9-base and imperfect 11-base repeats are enclosed by solid-line boxes.

sequences from several sections of the other full-length clones revealed that these retrotransposons fall into two discrete transposon families, Tf1 and Tf2. The LTR of Tf1-112 was sequenced and found to be 99% identical to Tf1-107 (Fig. 2b). The solo LTR found on plasmid pEE102 was also found to belong to the Tf1 family because its sequence was 91.3% identical to that of Tf1-107 (Fig. 2b). In contrast to the Tf1 elements, the closely related Tf2-111, Tf2-21, and Tf2-22 elements were found to have a different DNA sequence that was only 28% identical to the Tf1-107 DNA sequence within a 78-base region of the LTR and 61% identical over a region of 150 bases within the CA region but otherwise clearly related to the Tf1 sequence. Additional sequence data on Tf2-111 and the results of genomic DNA blot experiments described below indicate that the areas that differ drastically between the two transposon families include the central portions of the LTRs and a region extending from just downstream of the 5′ LTR to the middle of the putative capsid protein domain (Fig. 2a). Each family of Tf elements was found to occur within various independent *S. pombe* isolates. Interestingly, the solo LTR derived from 975, a strain that has no intact copies of Tf1 (see below), was a Tf1 LTR.

LTR features and target site duplications. The alignment of

Tf element LTRs in Fig. 2b indicates the presence of candidate TATA boxes, all of which were found to be identical to the TATA box of the *adh* gene, a highly expressed *S. pombe* gene (32). Also shown in Fig. 2b are the positions of the two 9-base repeats present at the beginning of each of the LTRs and imperfect 11-base repeats that are also upstream of the TATA boxes. The 11-base sequence, which is imperfect because the upstream copy in Tf1 has an extra thymidine residue inserted, is present only once in the Tf2 LTR. The position of these repeats with respect to the TATA boxes suggests that they play a role in Tf element transcription.

Most retroviruses and retrotransposons produce a 4- to 6-bp target site duplication upon integration of a new DNA copy into the host genome (2). Therefore, target site duplication indicates that transposition-mediated insertion of a retrotransposon has occurred. Sequence analysis of the DNA flanking two Tf1 elements, three Tf2 elements, and the solo Tf1 LTR indicated duplication of the five bases flanking the LTR sequences in all six cases (Fig. 2b). The duplicated sequences were all different from each other but were very A + T rich (87%). The observation that all of the Tf1 and Tf2 target sites sequenced contained 5-bp duplications strongly suggests that these elements are retrotransposons and integrate into the genome via typical transposition events. Because the closely related *S. cerevisiae* element Ty3 (and its solo LTR, the σ element) are always found 15 to 17 bp upstream of tRNA coding regions (16, 34), we examined the DNA flanking the Tf element insertion sites for tRNA-like sequences by using the program tRNA GENE SEARCH in the ANALYSEQ package (39). No such sequences were identified, suggesting that Tf1 and Tf2 do not have the unusual association with tRNA genes typical of Ty3.

Genomic distribution of Tf1 and Tf2 elements. We prepared a Tf1-specific hybridization probe from an *Eco*RI fragment (bases 564 to 1312) of Tf1-107 to probe genomic DNA blots of several diverse *S. pombe* strains. The results (Fig. 3a) indicate that in three wild *S. pombe* strains, the positions and copy numbers of Tf1 vary greatly. Strains NCYC 132 and CSIRY457 have at least 20 hybridizing bands and, judging from the uneven intensities of many of the bands in these lanes, probably contain 30 to 40 copies of Tf1. In contrast, Leupold strain 975 and its multiply auxotrophic derivative YHL211 show no hybridization signal. Figure 3b shows a DNA blot of an identical gel that was hybridized with a *Sna*BI-*Cla*I fragment from Tf2-111 that is Tf2 specific. The results of this blot demonstrate that although 975 and YHL211 have different genotypes and YHL211 was derived from 975 through multiple rounds of mutagenesis and backcrossing, both strains lack Tf1 whereas they contain numerous copies of Tf2. Moreover, these Tf2 copies appear to be identical in number and chromosomal location, suggesting that Tf2 is relatively stable and confirming that the genetic homogeneity of the Leupold strains extends to their transposable elements as well.

Tf element transcription. To determine whether any Tf1 copies are transcriptionally active, we hybridized the *Eco*RI probe of Tf1-107 that hybridizes specifically to Tf1 to an RNA blot; this revealed an intense 4.5-kb band in NCYC 132 and, as predicted, no signal in 975 (Fig. 3c). The length of this transcript is sufficient to extend from one LTR to the other, as in many LTR-containing retrotransposons (41). Figure 3d is the identical RNA blot probed with the Tf2-specific *Cla*I-*Sna*BI probe. Strain NCYC 132 produced a strong signal, whereas 975 produced a similar but slightly less intense signal. The blurriness of the 4.5-kb band in these

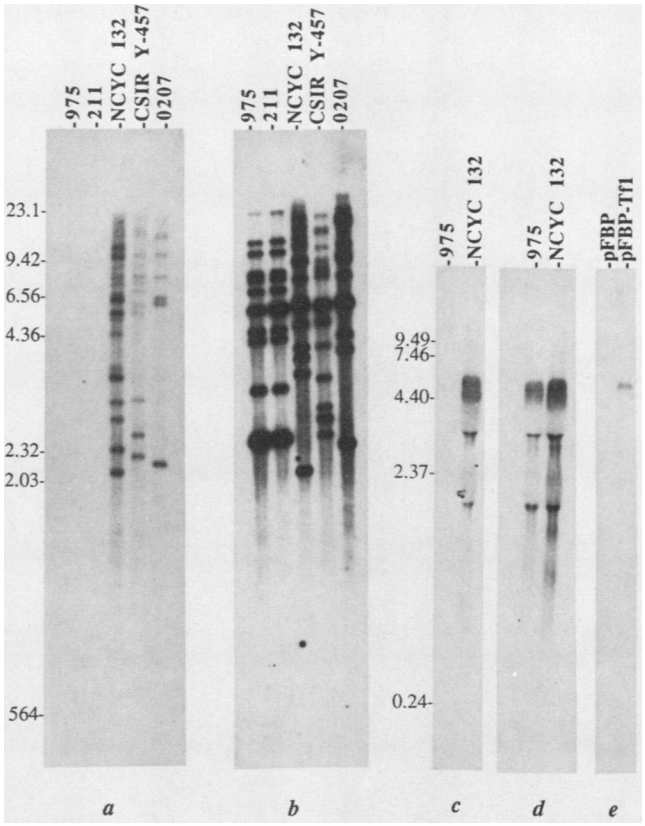

FIG. 3. S. pombe DNA and RNA blots hybridized with internal Tf1- and Tf2-specific probes. (a) A genomic blot was made with *Pst*I-cut DNA from strains 975, YHL211, NCYC 132, CSIR Y-457, and 0207 (43). All such *Pst*I fragments represent single copies of Tf1 because *Pst*I cuts Tf1 in half and the *Eco*RI fragment used as a Tf1-specific probe (nucleotides 564 to 1312) were derived exclusively from the 5′ half of the element (Fig. 1). (b) Identical to panel a, except that the filter was hybridized with a *Sna*BI-*Cla*I (bases 320 to 1340), Tf2-specific probe. (c) Total RNAs from *S. pombe* 975 and NCYC 132 were blotted and probed with the Tf1-specific *Eco*RI probe. Molecular size markers indicate that the size of the major species is approximately 4.5 kb. Total RNA staining with methylene blue showed that the levels of RNA loaded in all of the lanes were equivalent. (d) Same as panel c, except that the probe was the Tf2-specific *Sna*BI-*Cla*I probe. (e) RNA was derived from strains containing an *fbp* promoter plasmid (18) (pFBP) and this plasmid bearing a fusion between the *fbp* promoter and Tf1-107 (pFBP-Tf1).

RNA blots is probably due to the heterogeneity in structure among Tf elements, which are members of 15- to 50-copy multigene families. Tf1-107 was cloned behind the *S. pombe fbp* promoter by using the pCHY21 vector (18) and introduced into *ura4D-18* strain YHL232, a derivative of strain 975, by transformation. Indeed, the Tf1 transcripts produced in this strain are much more homogeneous than those seen in the *S. pombe* strains bearing multiple Tf1 copies (Fig. 3e). The two bands migrating at 3.3 and 1.7 kb apparently represent compression bands caused by the massive amounts of rRNA present in these total RNA preparations; they were not observed in poly(A)-selected RNA preparations (Fig. 4).

Some retrotransposons, like Ta1 of *Arabidopsis thaliana*, produce no detectable transcripts and appear to be dead because of mutations that inactivate essential regions of the sequence (D. F. Voytas, A. Konieczny, M. P. Cummings,

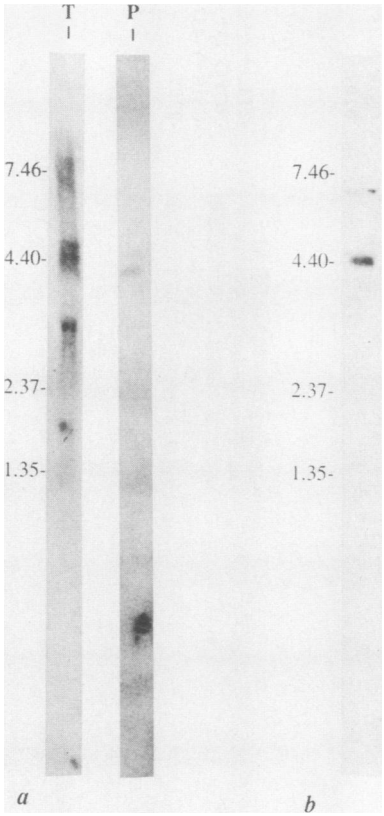

FIG. 4. Poly(A)-selected RNAs (a) T, Total RNA; P, poly(A)-selected RNA from wild strain NCYC 132. (b) Poly(A)-selected RNA from strain YHL351, a strain containing a high-copy-number plasmid bearing Tf1-107 and no chromosomal copies of Tf1. Molecular size standards are indicated in kilobases.

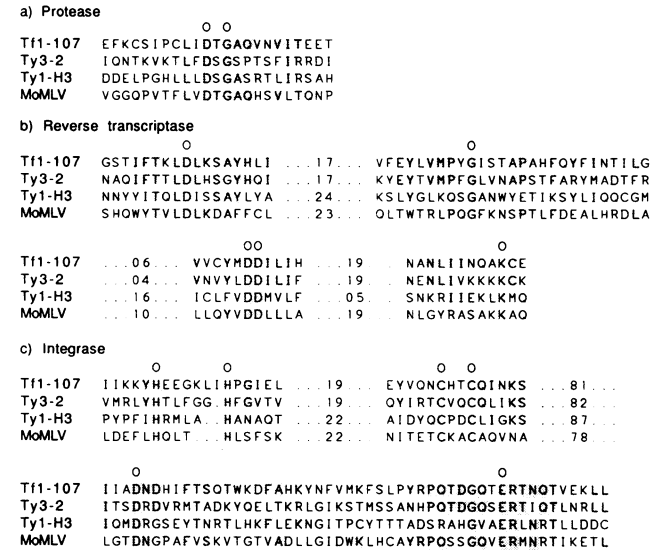

FIG. 5. Amino acid sequence comparisons of highly conserved sections of the PR, RT, RNase H, and IN domains of Tf1-107, Ty3-2 (16), Moloney murine leukemia virus (MoMLV) (36), and Ty1-912 (6). Shaded residues indicate sequences identical in Tf1-107 and at least one of the other elements shown. Alignments are based on those reported by several other researchers; a circle above a residue indicates identity among all or nearly all retrotransposons-retroviruses (8, 21, 30, 41a). For RT, the alignments are based on those of Poch et al. (30) and Xiong and Eickbush (in press).

and F. M. Ausubel, Genetics, in press). The fact that full-length Tf1 transcripts were observed demonstrates that at least some functionality was conserved and suggests that at least a subset of Tf1 copies are competent for transposition.

Tf elements belong to the *gypsy* family of retrotransposons. Comparison of the Tf1 RT, RNase H, PR, and IN domains with those of *pol* genes revealed extensive similarity at the amino acid level to elements in the *gypsy* retrotransposon family and, to a lesser extent, the Moloney family of retroviruses. The *gypsy* family contains members from many insect species (11, 20, 25, 33), as well as budding yeasts (16) and other fungi (26) and at least one plant species (38). The RT sequence in this family of elements is also closely related to the RTs of caulimoviruses, a family of plant DNA viruses that replicate via reverse transcription of an RNA intermediate (8). The element with the highest known level of amino acid identity to the RT domain of Tf1-107 is Ty3, a retrotransposon of *S. cerevisiae* which is 48% identical within the 67-amino-acid sequence shown (Fig. 4). By comparison, the amino acid sequence of RT from Moloney murine leukemia virus is only 25% identical to this conserved region of Tf1-107 RT.

Mode of gene expression. An intriguing aspect of Tf1 concerns its putative mode of gene expression. All retroviruses and retrotransposons studied to date have a special mechanism ensuring that they produce more *gag*-encoded than *pol*-encoded proteins. Most of these elements encode *gag* and *pol* as separate open reading frames; down modulation of *pol* expression relative to *gag* expression is usually effected by translational frameshifting (or a similar mechanism) that allows 10 to 20% efficient ribosomal readthrough from *gag* into *pol* (1). Tf1-107, on the other hand, contains but a single open reading frame. In addition, the DNA sequence of Tf1-112 from the middle of the CA section to the start of the RT domain contains an open reading frame, suggesting that most, if not all, Tf1 elements have a single open reading frame. *copia* proteins are also expressed in a single open reading frame, but in *copia*, there is a subgenomic 2.2-kb mRNA that is a spliced derivative of the full-length *copia* mRNA, which is much less abundant than the 2.2-kb mRNA in cells in which transposition is thought to be active (5, 28, 42). The 2.2-kb mRNA apparently encodes the Gag and PR proteins, whereas the full-length transcript encodes Gag-Pol proteins and presumably serves as genomic RNA. We observed no such abundant subgenomic Tf1 mRNA in *S. pombe* cells. Bands 2 and 3 from the top of Fig. 3c and d are presumed to be due to compression of Tf RNA degradation products caused by the superabundant rRNA in total RNA preparations. These bands do not represent specific subgenomic transcripts, as indicated by the following evidence. These minor bands do not appear on RNA blots of poly(A)-selected RNA probed with a Tf1-specific probe. This is true of RNAs isolated from both NCYC 132 and strain HL351, which is (Leupold) *ura4-D18* strain YHL232 transformed with plasmid pHL117-17, a high-copy-number plasmid bearing Tf1-107 (Fig. 4). We noted two minor transcripts (visible in the original autoradiogram but not obvious in the final figure), one slightly larger than the major transcript and one of approximately 3 kb in strain NCYC 132, whereas only one major species was seen in strain HL351. Since the latter strain contains no Tf1 elements, except for the one on the high-copy-number plasmid, and the transcript is evident, this experiment also shows that

Tf1-107 is transcriptionally active. We suspect that the minor species seen in the wild strain derive from defective or rearranged transposon copies. Subgenomic mRNAs from the *gag* region are also lacking in strain HL410 (Fig. 3e), a strain that has no chromosomal copies of Tf1 but bears a copy of Tf1-107 driven by the *S. pombe fbp* promoter (18). We conclude that Tf1 either dispenses with the apparent need to down modulate *pol* expression or uses a novel mechanism for down modulation.

The discovery of transposable elements in *S. pombe* will allow detailed investigation of transposition and related processes, such as recombination between dispersed repeats and activation of adjacent genes by retrotransposons, to be done with a genetically pliable eucaryotic microbe that has many biological similarities to the cells of higher-order organisms (31). In light of the mammalian-like qualities of transcription, RNA processing, DNA replication, chromosome condensation, and cell cycle control in *S. pombe* (15, 22, 23, 40), these transposons will provide an especially interesting system in which host functions for retrotransposition can be studied genetically, as has recently been done with *S. cerevisiae* (H. Xu and J. D. Boeke, Proc. Natl. Acad. Sci. USA, in press).

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