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Functional Genomics Reveals Relationships Between the Retrovirus-Like Ty1 Element and Its Host *Saccharomyces cerevisiae*

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ABSTRACT

Retroviruses and their relatives, the long terminal repeat (LTR) retrotransposons, carry out complex life cycles within the cells of their hosts. We have exploited a collection of gene deletion mutants developed by the *Saccharomyces* Genome Deletion Project to perform a functional genomics screen for host factors that influence the retrovirus-like Ty1 element in yeast. A total of 101 genes that presumably influence many different aspects of the Ty1 retrotransposition cycle were identified from our analysis of 4483 homozygous diploid deletion strains. Of the 101 identified mutants, 46 had significantly altered levels of Ty1 cDNA, whereas the remaining 55 mutants had normal levels of Ty1 cDNA. Thus, approximately half of the mutants apparently affected the early stages of retrotransposition leading up to the assembly of virus-like particles and cDNA replication, whereas the remaining half affected steps that occur after cDNA replication. Although most of the mutants retained the ability to target Ty1 integration to tRNA genes, 2 mutants had reduced levels of tRNA gene targeting. Over 25% of the gene products identified in this study were conserved in other organisms, suggesting that this collection of host factors can serve as a starting point for identifying host factors that influence LTR retroelements and retroviruses in other organisms. Overall, our data indicate that Ty1 requires a large number of cellular host factors to complete its retrotransposition cycle efficiently.

A relative of vertebrate retroviruses, Ty1 is a long terminal repeat (LTR) retrotransposon in the yeast *Saccharomyces cerevisiae* (Boeke and Sandmeyer 1991). Like retroviruses, Ty1 encodes homologs of Gag and Pol proteins, forms virus-like particles, and transposes through an RNA intermediate using reverse transcriptase (Boeke and Devine 1998). The Ty1 retrotransposition cycle is complex and begins in the nucleus with the transcription of full-length Ty1 elements. As the cycle progresses, virus-like particles are assembled in the cytoplasm, and, ultimately, double-stranded Ty1 cDNAs are generated from Ty1 mRNA templates. The cycle is completed with the integration of these newly synthesized cDNAs into chromosomal DNA in the nucleus.

The cDNA replication step of the retrotransposition cycle is thought to occur mainly within virus-like particles that are assembled in the cytoplasm (Garfinkel *et al.* 1985). Ty1 cDNAs are produced through the reverse transcription of Ty1 mRNA templates that are packaged into these particles (Garfinkel *et al.* 1985; Eichinger and Boeke 1988; Lauermann and Boeke 1997). Although the initial step of strong-stop synthesis has been studied in detail for Ty1 (Chapman *et al.* 1992; Lauermann and Boeke 1994; Keeney *et al.* 1995; Lauermann *et al.* 1995), relatively little is known about the remaining downstream steps of the cDNA replication process. These downstream steps are likely to include priming, strand-transfer, and processing events (Lauermann and Boeke 1997).

After the Ty1 cDNAs are fully replicated, they are imported into the nucleus where they are integrated into chromosomal DNA. This final integration step of the retrotransposition cycle occurs with a high degree of specificity in the yeast genome, and new retrotransposition events are targeted preferentially to tRNA genes and other genes that are transcribed by RNA polymerase III (RNA pol III; Ji *et al.* 1993; Devine and Boeke 1996). Some of the first evidence that Ty1 is targeted to tRNA genes came from the observation that most preexisting copies of Ty1 are located next to tRNA genes in the yeast genome (Ji *et al.* 1993; Devine and Boeke 1996; Kim *et al.* 1998). Additional support for this mechanism came from the observation that experimentally induced retrotransposition events are preferentially targeted to tRNA genes and other genes that are transcribed by RNA pol III (Ji *et al.* 1993; Devine and Boeke 1996; Boeke and Devine 1998). Targeting appears to require RNA pol III transcription (or factors associated with RNA pol III transcription) at the target gene, since tRNA gene mutations that diminish RNA pol III transcription

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also diminish Ty1 integration near the gene (Devine and Boeke 1996). Thus, specific RNA pol III transcription factors, or nearby chromatin factors, are likely to be involved in tRNA gene targeting.

Since the Ty1 retrotransposition cycle is complex and spans several intracellular compartments, it could be envisioned to involve a wide range of cellular host factors. Our goal was to take advantage of the excellent genomic resources that have become available in *S. cerevisiae* to systematically identify such host factors. To achieve this goal, we performed a functional genomics screen for host genes that influence Ty1 using the recently completed collection of gene deletion strains generated by the *Saccharomyces* Genome Deletion Project (Winzeler *et al.* 1999; Giaever *et al.* 2002). Our study has increased the number of host factors that are known to affect Ty1 and, together with previous studies, provides a more complete picture of the relationship between Ty1 and its yeast host.

MATERIALS AND METHODS

Plasmids and strains: The Ty1-*HIS3* donor plasmid pAR100 was constructed by first cloning a *Bam*HI/*Not*I fragment carrying a Gal-Ty1-*neo* insert (Devine and Boeke 1996) into the *Bam*HI/*Not*I sites of the plasmid pRS316 (Sikorski and Hieter 1989). A PCR cassette carrying the *HIS3* gene then was inserted at bases 6168–7080 of the Gal-Ty1-*neo* insert in both the forward and reverse orientations by homologous recombination in yeast (Kaiser *et al.* 1994). The *HIS3* cassettes were generated by PCR using the plasmid pRS403 (Sikorski and Hieter 1989) as a template and oligonucleotide primers with the following sequences: (SD516) 5′-TTACATTGCAC AAGATAAAAATATATCATCATGAACAATAAAACTAGATT GTACTGAGAGTGCAC-3′, (SD517) 5′-CGCCGTCCCGTCAA GTCAGCGTAATGCTCTGCCAGTGTTACAACCCTGTCGG GTATTTCACACCG-3′, (SD518) 5′-TTACATTGCACAAGAT AAAAATATATCATCATGAACAATAAAACTCTGTCGGGTA TTTCACACCG-3′, and (SD519) 5′-CGCCGTCCCGTCAAGT CAGCGTAATGCTCTGCCAGTGTTACAACCAGATTGTACT GAGAGTGCAC-3′. The *neo* gene of Gal-Ty1-*neo* was replaced by the *HIS3* gene using this strategy. Retrotransposition levels were similar for both constructs, and the reverse orientation construct, pAR100, was chosen for the screen (Figure 1A). As expected, this plasmid yielded His^+ cells upon growth on galactose, but did not produce His^+ colonies when grown only on glucose.

Haploid deletion strains were constructed from the yeast strain BY4741 (*MAT***a** *ura3*Δ*0 leu2*Δ*0 his3*Δ*1 met15*Δ*0*) using a *LEU2* cassette generated by PCR as described (Brachmann *et al.* 1998). Deletion primers were designed for each of the chromatin mutants listed in Table 1 using the 45-bp sequences flanking each open reading frame (ORF), and the A, B, C, and D primer sequences designed by the consortium (Winzeler *et al.* 1999; Giaever *et al.* 2002; http://www-deletions.stanford.edu). The *LEU2B* and *LEU2C* primers used in these studies had the following sequences: (*LEU2B*) 5′-CCGATGAAGCCTCCGTT ATCCC-3′ and (*LEU2C*) 5′-GTTCAGGTCTAACACTACCG-3′. Three independent strain isolates that passed all four PCR verification tests (A + B, A + *LEU2*B, C + D, and D + *LEU2*C) were constructed for each gene deletion. All three isolates then were tested in retrotransposition experiments and compared to the appropriate diploid deletion strain constructed by the consortium (with the same gene deleted). Similar results were obtained with the haploid and diploid deletion strains for each mutant (data not shown).

Ty1 retrotransposition assay: The complete set of homozygous gene deletion strains (release 2) was obtained from Research Genetics (Huntsville, AL). A complete list of the genes in the collection can be viewed at the Research Genetics website (ftp://ftp.resgen.com/pub/deletions/Homo_diploids_041902.txt). All strains in plates 301–349 were transformed individually with the pAR100 test plasmid in batches of 96 following the order established by the *Saccharomyces* Genome Deletion Project using a lithium acetate method adapted to 96-well culture boxes (Winzeler *et al.* 1999). Transformation reactions were plated on synthetic complete (SC) medium lacking uracil (SC-U). Three independent transformants were patched from single colonies onto SC-U medium to begin the retrotransposition assay. These plates next were replica plated onto SC-U medium containing 2% galactose and then incubated for 4 days at room temperature (24°) to induce retrotransposition. After 4 days of incubation, the SC-U/galactose plates were replica plated sequentially to: (i) SC-U medium containing glucose, (ii) yeast peptone dextrose medium, (iii) SC medium containing glucose and 1.2 g/liter 5-fluoroorotic acid (5-FOA), and (iv) SC minus histidine (SC-H) medium containing glucose (Figure 1B). Unless otherwise indicated, all plates were incubated overnight at 30° between each step. All media were prepared as outlined previously (Kaiser *et al.* 1994).

GAL1-lacZ*, *HIS3*, and YPG secondary screens:** Mutants that were positive in the initial screen were retested in three separate secondary assays to eliminate mutants that affected our assay rather than Ty1 retrotransposition itself. First, all mutants were tested in a *GAL1-lacZ* reporter assay to identify host genes that influenced the *GAL1* promoter used to induce retrotransposition from the Ty1 test plasmid. Positive and negative controls were used in all of the *GAL1:lacZ* assays to define the range of the assay. In each case, three controls were used: (i) the wild-type BY4743 strain containing the *GAL1:lacZ* plasmid (which served as a strong positive control), (ii) an isogenic *gal4* strain containing the *GAL1:lacZ* plasmid (which served as a weak positive control), and (iii) the BY4743 strain lacking the *GAL1:lacZ* plasmid (which served as a negative control). Each mutant was compared in triplicate to these controls, and mutants with reduced β-galactosidase activity were eliminated. Mutants with weak, moderate, and strong reductions in β-galactosidase activity were identified and eliminated with this approach. Only a small fraction of the mutant candidates affected the *GAL1* promoter as judged by the X-gal assay (Kaiser *et al.* 1994), including deletions in several *gal* genes, and these were eliminated from further consideration. A second test was performed to determine whether the *HIS3* marker in the test Ty1 element was functioning in each putative Ty1 mutant. Host mutants that affected marker function would not be expected to yield a His^+ phenotype after retrotransposition and would be indistinguishable from actual Ty1 mutants. Thus, we tested whether each mutant candidate (carrying a Ty1 test plasmid) could support a His^+ phenotype prior to the induction of retrotransposition by replica plating each strain to medium lacking histidine. A small number of strains were identified in this class, including strains carrying deletions in the known histidine biosynthesis genes (*his1*, *his2*, *his4*, *his5*, *his6*, and *his7*), and these were removed from further consideration. We also performed a third test to identify strains that could not support respiration efficiently and therefore might not utilize galactose efficiently. To perform this test, the initial SC-U plates that were used in the first step of the retrotransposition assay (above) were replica plated to yeast peptone glycerol (YPG) medium. Strains that grew more slowly on this medium compared to the wild-type BY4743 strain (*MATa**/α *ura3*Δ*0*/

ura3Δ0 leu2Δ0/leu2Δ0 his3Δ1/his3Δ1 met15Δ0/MET15 lys2Δ0/LYS2) were eliminated from further analysis.

Dilution assays: Retrotransposition levels were measured in triplicate for each mutant by plating serial dilutions of cells that had been induced for retrotransposition on medium that was selective for retrotransposition events (SC-H) and on two control media (SC and SC-U). Cells were scraped from the SC plus 5-FOA patches into water and diluted to an OD_{600} of 1. Twofold dilutions were prepared in 96-well microtiter dishes and then plated on all three media using a multichannel pipettor. The SC plate served as a control for adjusting the cells to an OD_{600} of 1, whereas the SC-U plate served as a control to ensure that the test plasmid had been eliminated successfully on the 5-FOA step. The number of cells growing at each dilution on the SC-H plate was compared to similar dilutions prepared from the wild-type strain and the fold change was estimated (rounding to the nearest twofold dilution). The three independent measurements were averaged to produce the final fold-change value reported (Figure 2C and supplemental Table 1 at http://www.genetics.org/supplemental/).

Targeting assays: The plasmid-based assay for tRNA gene targeting was performed as described previously using a Gal-Ty1-*neo* donor plasmid and the *SUF16* target plasmid pSD546 (Devine and Boeke 1996). Targeting was assessed in at least 36 independent patches. The recombinant frequencies were determined for each mutant in two independent experiments as described previously (Devine and Boeke 1996). The PCR assay for detecting Ty1 integration events upstream of glycine tRNA genes was similar to assays described previously (Smith *et al.* 1996; Scholes *et al.* 2001) and utilized the following primers: (SD609) 5′-GCGCAAGCCCGGAATCGAAC-3′ and (SD610) 5′-CAACAATTATCTCAACATTCACC-3′. The SD609 sequence was derived from the complementary strand of the *SUF16* tRNA gene, whereas the SD610 sequence was derived from the sense strand of the Ty1 LTR. The SD609 primer is homologous to 16 different glycine tRNA genes and is expected to detect Ty1 insertions upstream of all of these genes. The SD609 primer was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. The PCR then was performed with the SD609 and SD610 primers using the following cycling parameters: (i) 94° for 4 min; (ii) 28 cycles of 94° for 30 sec, 60° for 30 sec, and 72° for 30 sec; and (iii) a final extension at 72° for 10 min. A total of 150 His^+ colonies were independently pooled in duplicate for each mutant and analyzed using genomic DNA templates prepared from the pools. Following PCR, the reactions were precipitated with ethanol, washed with 70% ethanol, and resuspended in 10 μl water plus 10 μl of deionized formamide loading buffer. The samples were heated to 100° for 3 min and analyzed on a denaturing 6% polyacrylamide urea gel. The gel was dried and exposed to XAR5 film overnight at −80°.

Ty1 cDNA analysis: Ty1 cDNA was measured by Southern hybridization analysis after a 48-hr induction in medium containing galactose. DNA was isolated from duplicate pAR100 transformants and analyzed as follows. After measuring the DNA concentration of each sample with a spectrophotometer, 10 μg of DNA was digested with the restriction endonuclease *Afl*II (which cuts 2472 bp from the right end of Ty1-*HIS3* cDNA) and run on a 1% agarose gel. The DNA was transferred to a nylon membrane (Osmonics, Minnetonka, MN) and then hybridized to a 1.4-kb probe that spanned the full *HIS3* gene. Using this strategy, cDNA originating from the pAR100 donor plasmid was detected, but cDNA arising from genomic Ty1 copies was not detected. The *HIS3* probe also hybridized to the linearized donor plasmid pAR100 and the *his3Δ1* allele in the BY4743 strain background, thereby generating two additional bands in each lane (at 13 and 5 kb, respectively). These bands served as loading controls to ensure that equal amounts of DNA were analyzed in each lane. The prehybridization/hybridization buffer contained: 6× SSC, 0.01 M EDTA (pH 8.0), 5× Denhardt's solution, 0.5% SDS, and 100 μg/ml sheared, denatured salmon sperm DNA. The prehybridization, hybridization, and final wash steps were carried out at 65°. The washed membranes were exposed to XAR5 film and analyzed with a Fujix BAS1000 phosphoimager after exposing the membranes to phosphoimaging screens. Ty1 cDNA was measured in the duplicate samples by digital analysis of the scanned images, and the duplicates were averaged to obtain the final values reported. The Ty1 cDNA levels were considered to be altered from wild type if the average of the duplicate measurements was <50%, or >200%, of wild-type control cDNA levels.

RESULTS

Systematic analysis of Ty1 retrotransposition in the gene deletion collection: Our experimental strategy involved screening the collection of yeast deletion strains developed by the *Saccharomyces* Genome Deletion Project (Winzeler *et al.* 1999; Giaever *et al.* 2002) for mutants that affect the Ty1 retrotransposition cycle. An advantage of this approach is that all ~6200 yeast genes have been deleted in this single isogenic collection of knockout strains, allowing many genes to be tested in parallel for possible effects on a given process (in this case, Ty1 retrotransposition). Approximately 17% of the genes in yeast are "essential" and thus produce lethal phenotypes upon gene deletion (Winzeler *et al.* 1999; Giaever *et al.* 2002). However, the remaining 83% of gene knockouts are viable and therefore can be tested readily for additional phenotypes. Thus, we used a high-throughput, functional genomics approach to systematically test this latter class of viable yeast mutants to identify genes that affected the Ty1 retrotransposition cycle.

A donor plasmid carrying the Ty1 element was introduced separately into 4483 homozygous deletion strains using a 96-well transformation protocol, and each strain was tested for its ability to support Ty1 retrotransposition (see materials and methods). The modified Ty1 element used in these studies was placed under the control of the galactose-inducible *GAL1* promoter and was used to test retrotransposition as described previously (Boeke *et al.* 1985). The yeast *HIS3* gene was engineered into this test Ty1 element as a convenient marker for retrotransposition events in the *his3Δ1* genetic background of the knockout collection (Brachmann *et al.* 1998; Winzeler *et al.* 1999). Thus, if Ty1 transposed from the test plasmid into the yeast genome, it carried with it the *HIS3* gene and conferred a His^+ phenotype to otherwise His^- cells (Figure 1A).

Using this plasmid-based assay, deletion strains with significantly altered levels of Ty1 retrotransposition were identified readily from the knockout collection (Figure 1B). In fact, 2.3% of the yeast genes tested showed a Ty1 retrotransposition phenotype, for a total of 101 mutants in the collection of 4483. Most of the

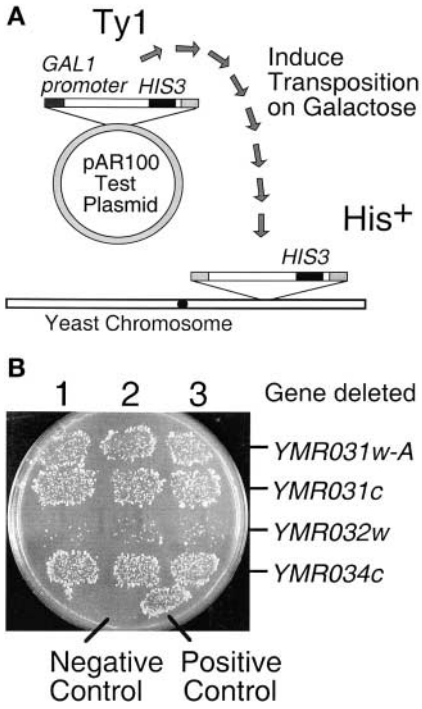

FIGURE 1.—Strategy for a functional genomics screen for genes that affect Ty1. (A) The test Ty1 plasmid depicted was introduced into each of the 4483 deletion strains and each strain was tested for its ability to support Ty1 retrotransposition. Retrotransposition was induced by growing cells carrying the test plasmid on galactose. If Ty1 transposed into the yeast genome, it carried with it the *HIS3* gene and conferred a His^+ phenotype. (B) An example of the results obtained in our screen. Four knockout strains (listed to the right of the plate) were tested in triplicate (listed 1–3 above the plate) on each SC-His plate (after inducing retrotransposition). Two controls were included on each plate. The negative control was the wild-type BY4743 strain (BRACHMANN *et al.* 1998; WINZELER *et al.* 1999) carrying the pRS316 plasmid (bottom left; SIKORSKI and HIETER 1989) and the positive control was the wild-type BY4743 strain carrying the pAR100 Ty1 test plasmid (bottom right). The positive control yielded a retrotransposition rate of ~1% under our test conditions, as judged by the appearance of His^+ cells. The *YMR032w* strain on this plate (third row from the top) showed a clear decrease in Ty1 retrotransposition (in triplicate), and all three patches showed decreased numbers of His^+ cells. Twenty-four plates such as the one shown were used to test each box of 96 deletion strains.

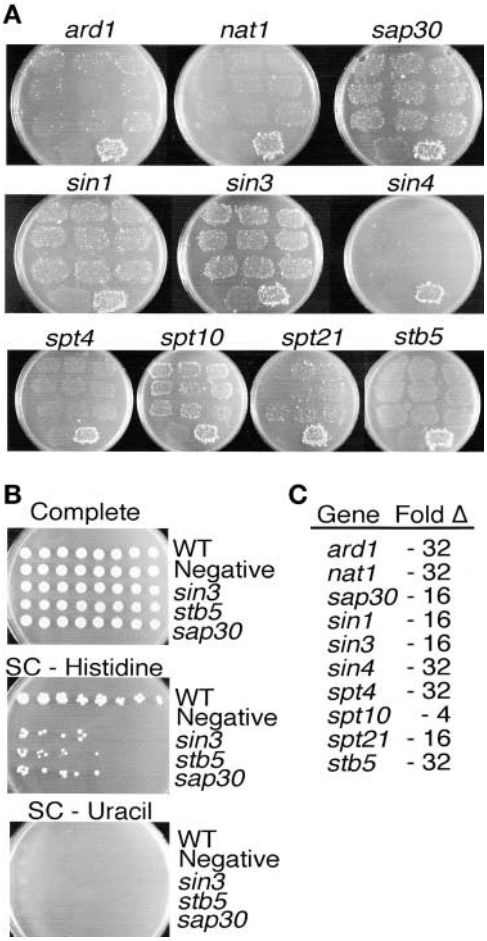

FIGURE 2.—Retrotransposition data for the chromatin mutants. (A) Retrotransposition results are shown for the chromatin mutants identified in our screen. On each plate, the top row shows retrotransposition data from the original three transformants, the second row from the top shows retrotransposition in cells from the frozen stocks of those original three transformants, and the third row shows retrotransposition in cells of the three retransformants. Negative and positive controls are shown at the bottom of each plate as described for Figure 1B. Equivalent results were obtained with knockout strains generated using a *LEU2* deletion cassette to delete the same genes in the BY4741 strain background (data not shown). (B) An example of a quantitative retrotransposition assay. Cells were scraped from the SC plus 5-FOA plate, diluted to an OD_{600} of 1, and plated in twofold serial dilutions (from left to right). The SC-Histidine plate measures the level of retrotransposition in each strain. The SC medium serves as a control for adjusting the cells to an OD_{600} of 1 and plating an equivalent number of cells in each row, whereas the SC-Uracil plate serves as a control to ensure that the test plasmid had been eliminated successfully at the 5-FOA step. (C) The fold changes for the chromatin mutants. Each mutant was tested in triplicate using the assay outlined in B. The value reported represents the average of the three measurements. The fold changes for all of our mutants are listed in supplemental Table 1 at http://www.genetics.org/supplemental/and also are available at our website database (http://biochemistry.emory.edu/ty1).

mutants had decreased levels of retrotransposition (99/101 or 98%), whereas only 2/101 (2%) had increased levels (*yml105c* and *yol159c*). Retrotransposition mutants were independently confirmed by retransforming each strain with the Ty1 plasmid and retesting it along with the original transformants and frozen stocks of the original transformants. The results of these comparisons were remarkably consistent (Figure 2A).

Three classes of genes are expected to affect the Gal-Ty1 assay rather than affect retrotransposition itself: (i) genes that affect *GAL1* promoter function, (ii) genes that affect marker gene function (in this case, *HIS3*), and (iii) genes that affect respiration and thus affect galactose utilization. Therefore, all initial mutant candidates were subjected to three secondary tests to eliminate host genes that affected these aspects of our assay rather than Ty1 retrotransposition itself (see MATERIALS AND METHODS). As expected, *gal* and *his* mutants were identified in these secondary screens, along with mu-

TABLE 1

Deletion strains with moderate or strong changes in Ty1 retrotransposition

Group/no. of genes	Genes deleted
Chromatin (10)	*ARD1, NAT1, SAP30, SIN1 (SPT2), SIN3, SIN4, SPT4, SPT10, SPT21, STB5*
DNA repair (4)	*APN1, MMS22, RAD52, XRS2*
Miscellaneous (27)	*APG17, APL5, BEM1, BUD6, CHO2, CYK3, DCC1, ERV14, FYV3, HOF1 (CYK2), JNM1, KCS1, KRE24, MAD2, MFT1, PAT1, NUM1, SCP160, SDF1, SEC22, SEC65, SMI1, SWA2, TPM1, TPS2, VPH1, VPS9*
Nuclear transport (2)	*NUP84, NUP133*
Protein folding/modification (8)	*CPR7, DBF2, DOA4, MCK1, NAT3, PFD1, SSE1, TCI1*
Ribosomes/translation (9)	*DBP3, RPL6A, RPL14A, RPL16B, RPL19B, RPL20B, RPL21B, RPP1A, RPS10A*
RNA metabolism (8)	*CBC2, DBR1, LEA1, LSM1, NOP12, RIT1, STO1 (CBC1), YDL033c*
Transcription (10)	*CTK1, DEP1, HAC1, PHO23, POP2, RPA49, RTF1, SRB8, SSN2, SUB1*
Transcription elongation (7)	*ELP2, ELP3, ELP4, ELP6, IKI3 (ELP1), KTI12, THP2*
Unknown (16)	*YBR077c, YDL115c, YDR496c, YFL032w, YGL250w, YGR064w, YKL053c-A, YLR052w, YLR322w, YML010c-B, YNL226w, YNL228w, YNL295w, YOL159c, YOR292c, YPL080c*

tants that grew poorly on YPG medium. Growth on YPG medium was tested to identify mutants that do not support respiration and thus might not utilize galactose efficiently in our retrotransposition assay. Although all of these mutants represented unwanted by-products of our genomic screen, they were fully expected to affect our assay and thus served as excellent internal controls for the accounting system of the knockout collection. No other sources of background are known to affect the specific Gal-Ty1 assay used in these studies. Therefore, the remaining 101 *T*y1 *h*ost *f*actor (*thf*) mutants that passed these secondary tests were considered to have Ty1 retrotransposition phenotypes. These 101 genes are known to participate in a wide range of cellular processes and could be classified into 10 major groups on the basis of the known functions of the encoded proteins (Table 1). The data for the chromatin mutants are shown in Figure 2 and the full set of data for all of the mutants in Table 1 can be viewed at our website database (http://biochemistry.emory.edu/ty1).

To more precisely measure the degree of change in each retrotransposition mutant, we performed quantitative retrotransposition assays on all of the mutants listed in Table 1 (see also MATERIALS AND METHODS). The results of these assays confirmed and extended the initial observations from the patch assays. Fifty of the mutants produced "moderate" (three- to eightfold) changes in retrotransposition levels and 51 mutants produced "strong" (greater than eightfold) changes in retrotransposition levels (supplemental Table 1 at http://www.genetics.org/supplemental/). An example of the assay is shown in Figure 2, B and C. Additional mutants also were identified, but strains with fewer than threefold changes were omitted from the final collection.

Targeted integration: The patch assays described above measure the overall levels of retrotransposition that occur in a given strain. Since Ty1 is known to integrate preferentially near tRNA genes, we also wanted to test whether any of the mutant strains in our collection affected the targeting step of the retrotransposition cycle. To do this, we needed to use an assay that would differentiate between mutants that affected any of the upstream steps of the retrotransposition cycle (and therefore did not produce integration events) *vs.* mutants that affected the integration step itself. Thus, we used a plasmid-based targeting assay to measure the frequency of Ty1 integration near a specific tRNA gene (*SUF16*) on a target plasmid. We previously found that the *SUF16* tRNA gene served as a high-frequency target in this assay and that the resulting integration events were targeted to the region upstream of the *SUF16* gene (DEVINE and BOEKE 1996). Similar results were obtained with the wild-type BY4741 strain background used in these studies, with an average targeting frequency of 3.97×10^{-4} (Table 2).

It is important to note that these *SUF16* targeting assays were performed exclusively with cells that contained at least one new retrotransposition event (*i.e.*, grew on medium that was selective for new transposon insertions). As a consequence, retrotransposition events that were stalled at any of the upstream steps of the retrotransposition cycle were eliminated from this analysis. Each of the new transposon insertions that occurred in these cells was targeted to either the *SUF16* plasmid or the yeast genome. If the new insertion was targeted to the *SUF16* plasmid, then a new recombinant plasmid that carried a Ty1 element was formed. If, instead, the new insertion was targeted to the yeast genome, then the *SUF16* plasmid remained unchanged. The *SUF16* targeting frequency then was determined by rescuing all of the *SUF16* target plasmids from the cells tested and by examining the fraction of target plasmids with new Ty1 insertions. This allowed us to directly measure whether the *SUF16* plasmid was targeted at the normal frequency by Ty1 in a given strain.

We hypothesized that chromatin factors might affect tRNA gene targeting because they are physically associated with chromosomes. Such factors also are frequently

TABLE 2

Ty1 targeting frequencies with a *SUF16* tRNA target plasmid

Strain	Experiment	Targeting frequency	Fold change	Average
Wild type (BY4741)	1	5.40×10^{-4}	—	
	2	4.58×10^{-4}	—	
	3	1.92×10^{-4}	—	
		Average: 3.97×10^{-4}	1	1
ard1	1	2.02×10^{-4}	−1.96	
	2	1.90×10^{-4}	−2.09	−2.0
nat1	1	1.10×10^{-4}	−3.61	
	2	1.80×10^{-4}	−2.21	−2.7
sap30	1	3.38×10^{-4}	−1.18	
	2	5.36×10^{-4}	+1.35	1.1
sin1	1	7.66×10^{-4}	+1.93	
	2	8.24×10^{-4}	+2.07	2.0
sin3	1	1.32×10^{-4}	−3.01	
	2	7.41×10^{-4}	+1.87	1.1
sin4	1	3.30×10^{-4}	−1.20	
	2	11.6×10^{-4}	+2.92	1.9
spt4	1	2.49×10^{-4}	−1.59	
	2	5.79×10^{-4}	+1.46	1.0
spt10	1	4.75×10^{-4}	+1.20	
	2	9.89×10^{-4}	+2.49	1.8
spt21	1	Not done	—	—
stb5	1	3.30×10^{-4}	−1.20	
	2	1.37×10^{-4}	−2.90	−1.7

involved in regulating the accessibility of DNA (Winston *et al.* 1984; Sternberg *et al.* 1987; Aparicio *et al.* 1991; Park and Szostak 1992; Dollard *et al.* 1994; Kasten and Stillman 1997; Zhang *et al.* 1998). Surprisingly, we determined that the targeting frequencies in our chromatin mutants were not altered significantly from the wild-type control strain (Table 2). Most of the chromatin mutants had targeting frequencies that were within about twofold of the wild-type control strain (Table 2). The *ard1*, *nat1*, and *sin1* strains had the largest average changes (2-fold, 2.7-fold, and 2-fold, respectively; Table 2); however, these relatively small changes could not account for the 16- to 32-fold decreases in retrotransposition observed in the patch assays performed with these mutants (which measure overall retrotransposition levels; Figure 2). Ty1 insertion sites also were mapped in each mutant, and all of the insertions examined were found upstream of the *SUF16* tRNA gene as expected for normal targeting (data not shown). Thus, *SUF16* targeting does not appear to be affected in any of the chromatin mutants tested, and a different step of the retrotransposition cycle is likely to be affected in these mutants.

We next examined whether tRNA gene targeting was affected in any of the remaining mutants in our collection. We developed a more streamlined approach to rapidly examine all of the mutants in our collection using a PCR-based targeting assay that is similar to assays described previously (Smith *et al.* 1996; Scholes *et al.* 2001; Figure 3A). This assay was designed to simultaneously detect Ty1 integration events upstream of 16 different glycine tRNA genes (see materials and methods). As for the *SUF16* plasmid targeting assay described above (Table 2), we limited our analysis to cells that contained at least one new retrotransposition event.

Ty1 generally integrates within an "integration zone" that begins ~80 bp upstream of a tRNA gene and extends several hundred base pairs in the upstream direction (Devine and Boeke 1996). Our PCR assay detected integration events within this zone, as expected, with optimal detection between positions −80 and −300 relative to the transcription start sites of the target genes. Two PCR bands were detected in all of the samples analyzed (Figure 3B). These bands presumably were caused by preexisting copies of Ty1 in the genome, because they were detected in strains that were not induced for retrotransposition (Figure 3B, duplicate lanes labeled −C). However, wild-type BY4743 cells that were induced for retrotransposition on galactose showed additional PCR bands that were caused by new integration events upstream of the target genes (Figure 3B, +C1 and +C2). The assay detected PCR products from pools containing as few as 100 retrotransposition events (His^+ colonies) in the wild-type BY4743 strain (Figure 3B, +C1), and we performed all of our analyses with duplicate pools of 150 independent retrotransposition events (His^+ colonies).

All of the host factor mutants in our collection were

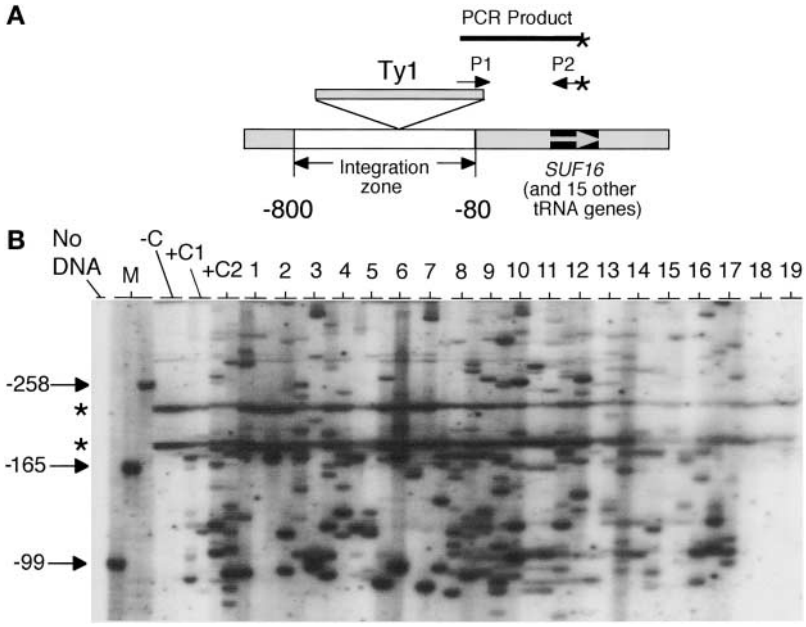

FIGURE 3.—*SUF16* PCR targeting analysis. A PCR-based assay was used to measure tRNA gene targeting. (A) Diagram of the assay. If Ty1 integrates upstream of any of 16 different glycine tRNA genes, a PCR product is formed. One of the primers (depicted as P2) is end-labeled with [γ-^{32}P]ATP. The PCR products are run on a 6% denaturing polyacrylamide gel and exposed to film for visualization. The upstream integration zone extends from positions −80 to −800 bp relative to the transcription start sites of the target genes. Our gels optimally detected insertions in the −80- to −300-bp region (due to gel resolution). (B) The results of a PCR assay with 19 mutants and controls. The first lane shows a PCR reaction without added DNA (labeled No DNA). The next three lanes show PCR products produced from control recombinant plasmids containing Ty1 insertions upstream of the *SUF16* tRNA gene (at positions −99, −165, and −258 relative to the transcription start site of *SUF16*). The next two duplicate lanes, labeled −C, depict negative controls in which cells were grown only on glucose prior to the PCR. Note the two PCR bands (labeled with stars) that were present in all of the samples tested and presumably were caused by preexisting Ty1 elements. The next two lanes, labeled +C1, show the results with duplicate pools of 100 retrotransposition events in the wild-type BY4743 strain. The next two lanes, labeled +C2, show the results with duplicate pools of 300 retrotransposition events in the wild-type BY4743 strain. The remaining lanes show the results with duplicate pools of 150 retrotransposition events in selected mutant strains. The duplicate lanes are numbered 1–19 and represent the following mutants: duplicate 1, *ard1*; 2, *nat1*; 3, *sap30*; 4, *sin1*; 5, *sin3*; 6, *sin4*; 7, *spt4*; 8, *spt10*; 9, *spt21*; 10, *stb5*; 11, *apn1*; 12, *mms22*; 13, *rad52*; 14, *xrs2*; 15, *cbc1*; 16, *cbc2*; 17, *dbr1*; 18, *rit1*; and 19, *ctk1*. All of the mutants except for *rit1* (duplicate 18) and *ctk1* (duplicate 19) retained the ability to target tRNA genes. Both *rit1* and *ctk1* lacked new PCR bands but produced the two bands that were caused by preexisting Ty1 copies (compare −C with duplicates 18 and 19). We previously observed that Ty1 integrates with a periodicity upstream of *SUF16* due to positioned nucleosomes assembled in the upstream region (BOEKE and DEVINE 1998; unpublished data). This pattern is difficult to discern with the small number of events assayed here, but becomes evident when more events are assayed (not shown).

tested for tRNA gene targeting using this PCR assay. The chromatin mutants first were reexamined, and, as expected, these mutants supported normal levels of targeting (Figure 3B, mutants 1–10). These results were consistent with the data obtained with the *SUF16* plasmid-based assay (Table 2) and allowed us to calibrate the two assays. Although some variation in the number of PCR bands was observed between duplicate samples, approximately two to eight PCR bands were observed in most pools (Figure 3 and data not shown). These bands do not necessarily represent individual retrotransposition events. However, the heterogeneity in band patterns among different strains suggests that most bands probably do represent individual events. Almost all of the 101 host factor mutants examined in our collection had results that were equivalent to those shown in Figure 3B, and PCR products were detected in most of the mutants (Figure 3 and data not shown). However, two mutants, *rit1* and *ctk1*, lacked new PCR products in both of the duplicate pools examined in three completely separate experiments (Figure 3, duplicate lanes 18 and 19, and data not shown). The two ubiquitous PCR bands present in all samples (and presumably caused by preexisting Ty1 copies) were present in *rit1* and *ctk1* (Figure 3). Therefore, technical failure of the PCR can be ruled out as a possible cause of the *rit1* and *ctk1* results. Thus, although the majority of the host factor mutants retained the ability to target Ty1 integration to tRNA genes, two mutants, *rit1* and *ctk1*, displayed diminished levels of targeting. The Rit1 protein is an ADP ribosylase known to modify methionine tRNAs, whereas the Ctk1 protein is a protein kinase. These two proteins are likely to affect tRNA gene targeting through different mechanisms (see DISCUSSION).

The steady-state levels of Ty1 cDNA are altered in many of the host factor mutants: We next determined whether the host factor mutants in our collection produced normal levels of Ty1 cDNA. Because double-stranded Ty1 cDNA is produced approximately midway through the retrotransposition cycle, it is a convenient measure of how far the retrotransposition cycle has progressed in a given mutant. Mutants with diminished levels of cDNA would be considered to affect the "early" steps of retrotransposition leading up to virus-like particle assembly and cDNA replication, whereas mutants with normal levels of cDNA would be considered to affect the "late" steps of retrotransposition that occur after cDNA production.

Interestingly, 9 of the 10 chromatin mutants examined were found to have significantly decreased levels of Ty1 cDNA compared to the wild-type BY4743 control strain (Figure 4A). Therefore, rather than affecting tRNA

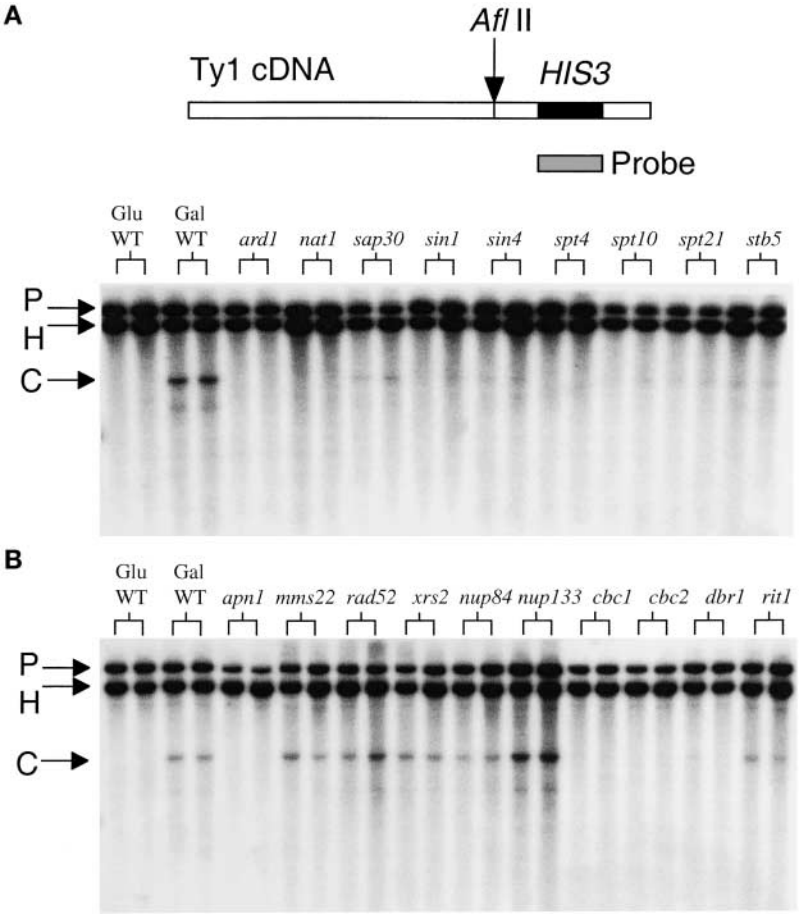

FIGURE 4.—Ty1 cDNA analysis by Southern hybridization. Ty1 cDNA was examined in duplicate for each strain by Southern hybridization analysis. Ty1 cDNA generated from the pAR100 donor plasmid produced a band at 2.5 kb (labeled C). Two other bands also hybridized to the probe: the donor plasmid (labeled P at 13 kb) and the *his3Δ1* allele of the *HIS3* gene (labeled H at 5 kb). (A) Southern blot of the chromatin mutants with decreased levels of cDNA. In each case, two independent transformants (carrying the pAR100 plasmid) were analyzed in duplicate lanes. The first two lanes show negative controls that were grown on glucose. Note the absence of the cDNA band. The next two lanes show the positive controls that were induced on galactose. A cDNA band at 2.5 kb is produced in both of the duplicates. The next lanes show the nine chromatin mutants that were found to have cDNA decreases in duplicate. The remaining chromatin mutant, *sin3*, had normal levels of cDNA (not shown). The hybridized membranes were exposed to XAR5 film to generate the figure shown and also were scanned using a Fujix BAS1000 phosphoimager after exposure to phosphoimager screens. The cDNA values were obtained by digital analysis of the scanned images, and the two cDNA measurements were averaged to produce the final value reported. The averages for the cDNA data shown (relative to the positive controls) were: −C, 0%; +C, 100%; *ard1*, 12.3%; *nat1*, 22.9%; *sap30*, 28.7%; *sin1*, 20.1%; *sin4*, 22.2%; *spt4*, 16.5%; *spt10*, 15.9%; *spt21*, 12%; and *stb5*, 14.6%. Similar results were obtained in a duplicate experiment (data not shown). (B) Southern blot of additional mutants performed as described in A. The averages for the cDNA measurements (relative to the positive controls) were: −C, 0%; +C, 100%; *apn1*, 16.9%; *mms22*, 112.1%; *rad52*, 161%; *xrs2*, 112.3%; *nup84*, 103.6%; *nup133*, 373.5%; *cbc1*, 12.1%; *cbc2*, 18.4%; *dbr1*, 18.1%; and *rit1*, 70.5%. We developed criteria for classifying the mutants as having "altered" or "normal" levels of cDNA on the basis of such results. Strains with <50% or >200% of wild-type cDNA levels (plus or minus twofold of wild type) were considered to be altered. All other strains were considered to have normal cDNA levels that were indistinguishable from wild type. These criteria were used to generate the data shown in Table 3.

gene targeting, as we had originally postulated (Table 2), most of the chromatin mutants affected the production (or turnover) of Ty1 cDNA. Upon analyzing all of the mutants in our collection in duplicate by Southern analysis, we found a total of 44 strains with decreased levels of Ty1 cDNA (<50% of wild-type levels) and 2 mutants with increased levels of cDNA (>200% of wild-type levels; Figure 4 and Table 3). The remaining 55 mutants had normal levels of cDNA (between 50 and 200% of wild-type levels; Figure 4 and data not shown). Thus, almost half of the 101 mutants identified in our study affected the early steps of the Ty1 retrotransposition cycle leading up to the formation of virus-like particles and cDNA replication, whereas the remaining half affected the later steps that occur after cDNA replication.

DISCUSSION

We have exploited a collection of gene deletion mutants developed by the *Saccharomyces* Genome Deletion Project (WINZELER *et al.* 1999; GIAEVER *et al.* 2002) to perform a functional genomics screen for host factors that influence the endogenous retrovirus-like Ty1 element in yeast. A total of 101 genes that presumably influence many different aspects of the Ty1 retrotransposition cycle were identified from our analysis of 4483 homozygous deletion strains. These genes are known to participate in a wide range of cellular processes and could be classified into 10 major groups on the basis of the known functions of the encoded proteins. Our study has increased the number of host factors that are known to affect Ty1 and provides a foundation for many future studies on the retrotransposition cycle.

Host factor mutants with altered levels of Ty1 cDNA: Forty-six of the mutants identified in our screen had altered levels of Ty1 cDNA as measured by Southern hybridization analysis (Figure 4 and Table 3). Of the 46 mutants, 44 had decreased levels of cDNA, whereas 2 mutants had increased levels of cDNA. Since we eliminated mutants that affected the *GAL1* promoter used in our Gal-Ty1 donor plasmid, none of the mutants is expected to affect the initial transcription step of the retrotransposition cycle in this system (Figure 5, step

TABLE 3

Mutants with altered cDNA levels

Strain	cDNA level (% BY4743)	Strain	cDNA level (% BY4743)
Control		Protein folding/modification	
BY4743	100	*doa4*	20.1
Chromatin		*mck1*	7.1
ard1	12.3	*nat3*	2.9
nat1	22.9	*sse1*	20.1
sap30	28.7	Ribosomes/translation	
sin1	20.1	*rpl6a*	12.5
sin4	22.2	*rpl19b*	24.2
spt4	16.5	*rpl20b*	16.2
spt10	15.9	*rps10a*	6.1
spt21	12.0	RNA metabolism	
stb5	14.6	*cbc1*	12.1
DNA repair		*cbc2*	18.4
apn1	16.9	*dbr1*	18.1
Nuclear transport		*lsm1*	13.6
nup133	373.5	Transcription	
Miscellaneous		*ctk1*	10.5
bem1	19.6	*pop2*	12.9
fyv3	15.5	*rtf1*	9.4
hof1	5.2	*rpa49*	8.1
jnm1	25.0	*ssn2*	21.7
kcs1	9.9	Transcription elongation	
mft1	15.6	*thp2*	16.6
num1	15.1	Unknown	
pat1	8.8	*ydr496c*	9.7
scp160	36.3	*yor292c*	12.1
sec22	14.7	*ynl226w*	22.3
tps2	18.3	*ynl228w*	19.6
vps9	41.1	*yol159c*	351.1

1). However, several subsequent steps of the cycle must be completed before any Ty1 cDNA can be replicated, and mutants with diminished levels of cDNA could be deficient in any of these steps. Such steps include: (i) the initial processing of Ty1 mRNA in the nucleus (Figure 5, step 2), (ii) the export of Ty1 mRNA from the nucleus (Figure 5, step 3), (iii) the translation of Ty1 proteins on ribosomes (Figure 5, step 4), and (iv) the assembly of virus-like particles in the cytoplasm (Figure 5, step 5). The cDNA levels might also be affected by changes in the rate of cDNA replication or turnover.

Of the 10 chromatin mutants examined in our study, 9 produced diminished levels of Ty1 cDNA compared to the BY4743 wild-type strain (Figure 4A). One possible model to explain these results would be that these chromatin factors normally play an important role in protecting the Ty1 cDNA from degradation by nucleases. In the absence of these chromatin factors, the Ty1 cDNA is more vulnerable to nuclease digestion, and thus Ty1 cDNA levels are decreased in such chromatin mutants. This model predicts the existence of an important chromatinized cDNA intermediate that is necessary for retrotransposition. An alternative model would be that these chromatin factors regulate the expression of other genes that, in turn, affect cDNA replication or turnover. Such genes might include some of the other "early" genes identified in our study (Table 1). Additional studies will be required to differentiate between these (and perhaps other) models.

A number of other mutants in our collection also displayed decreased levels of cDNA and thus appear to affect early steps of the retrotransposition cycle. Within the RNA metabolism group, for example, both the *cbc1* and *cbc2* mutants had reduced levels of Ty1 cDNA (Figure 4B). The Cbc1 and Cbc2 proteins form a "cap binding complex" that binds to the cap structure of cellular mRNAs (Fortes *et al.* 1999). Therefore, Cbc1 and Cbc2 are likely to affect retrotransposition by binding to either Ty1 mRNA or other cellular mRNAs that affect retrotransposition. Other mutants in the RNA metabolism group, such as *dbr1*, also had decreased levels of Ty1 cDNA (Figure 4B), consistent with previous reports (Karst *et al.* 2000). The *lsm1* mutant in this group likewise had decreased levels of cDNA (Table 3). In contrast, the remaining four mutants within the RNA metabolism group had normal levels of cDNA (Figure 4B and data not shown).

Host factor mutants with normal levels of Ty1 cDNA: We also identified 55 mutants that had normal levels of Ty1 cDNA (within a range of plus or minus twofold

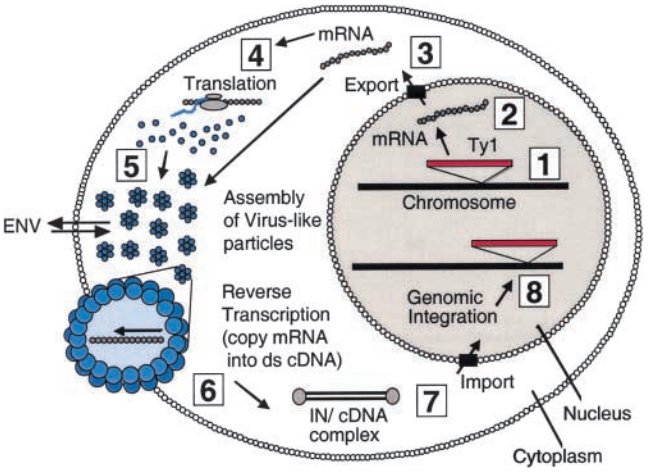

FIGURE 5.—A model for Ty1 host factor function. A model for the Ty1 retrotransposition cycle is depicted. The cycle begins with the transcription of Ty1 elements in the nucleus (step 1). Ty1 mRNAs are then processed and exported to the cytoplasm (steps 2 and 3). The mRNAs are next translated to produce Ty1 Gag and Pol proteins (step 4). Ty1 virus-like particles are assembled in the cytoplasm, and Ty1 mRNAs are packaged into these particles (step 5). The arrows exiting and entering the cell indicate the point at which retroviruses with envelope (ENV) genes can exit and infect a new cell. The Ty1 mRNAs next are copied into double-stranded (ds) cDNAs within the virus-like particles using reverse transcriptase (step 6). The cDNAs and Ty1 integrase (IN) then are imported back into the nucleus (step 7). The cDNAs finally are integrated into chromosomal DNA (step 8). Additional steps are likely to occur as well. For example, it is unclear how the Ty1 mRNA is directed to the ribosomes *vs.* the virus-like particles. Perhaps there is a specific mechanism for ensuring that the Ty1 mRNA arrives at ribosomes and virus-like particles after step 3. It is also unclear how the newly replicated cDNAs and integrase escape virus-like particles, suggesting that there may be a particle disassembly step between steps 6 and 7. It is also unclear how the Ty1 cDNA and integrase travel from the virus-like particle to the exterior of the nucleus, suggesting that there may be a cellular trafficking step between steps 6 and 7. DNA repair steps may occur during or following cDNA replication (step 6), as well as after integration (step 8). Most of the genes listed in Table 1 encode proteins that could be envisioned to function at one or more of the steps depicted.

of the wild-type control levels) as judged by Southern analysis (Figure 4 and data not shown). These mutants are likely to affect one or more of the "late" steps of retrotransposition that occur after the production of cDNA. One of the first steps that must occur after cDNA replication is the nuclear localization of the newly replicated Ty1 cDNA and integrase. Although it is presently unclear as to how the 6-kb Ty1 cDNA enters the nucleus, Ty1 integrase has a nuclear localization sequence that is required for retrotransposition (KENNA *et al.* 1998; MOORE *et al.* 1998). Therefore, integrase enters the nucleus using the normal nuclear import machinery. Two known nuclear pore mutants, *nup84* and *nup133*, that might affect this step of the retrotranposition cycle were identified in our screen. In support of this model, the *nup84* strain has normal levels of cDNA, indicating that it affects a late step of retrotransposition (Figure 4B). The *nup133* mutant has increased levels of Ty1 cDNA that, in principle, could be caused by the accumulation of cDNA in the cytoplasm in the absence of efficient nuclear transport (Figure 4B). Finally, the *sin3* mutant identified in our study may also affect the nuclear localization of Ty1 components, since *sin3* affects the nuclear import step of Tf1 retrotransposition in *Schizosaccharomyces pombe* (DANG *et al.* 1999).

After entering the nucleus, the cDNA is integrated into chromosomal DNA, primarily near tRNA genes (Figure 5, step 8). Despite the large number of host factors identified in our screen, only two factors that affected tRNA gene targeting were identified (see below). A likely explanation for this seemingly small number of targeting mutants is that we examined only the nonessential yeast genes in our study. Because most of the RNA pol III transcription factors are encoded by essential genes, it is likely that we missed at least some targeting factors by focusing only on nonessential yeast genes. Therefore, additional screens (focused on essential genes) will be necessary to identify all of the host factors involved in targeting.

After cDNA integration, some level of DNA repair is likely to be required at the integration site, and perhaps at other sites in the yeast genome, to repair damaged DNA created during retrotransposition. Four DNA repair mutants were identified in our study. Three of the DNA repair mutants, *mms22*, *rad52*, and *xrs2*, had normal levels of cDNA (Figure 4B) and therefore affected late steps of the retrotransposition cycle. Such factors could be involved in repairing chromosomal DNA damage at integration sites or elsewhere in the genome. The remaining mutant, *apn1*, had significantly decreased levels of cDNA and thus affected an early step of the retrotransposition cycle (Figure 4B). The Apn1 protein is an apurinic/apyrimidinic endonuclease that cleaves DNA at abasic sites to facilitate DNA repair. One possible explanation for Apn1 function would be that it is involved in cDNA repair prior to integration. If the cDNA were not repaired properly in an *apn1* mutant, perhaps the cDNA would be targeted for degradation.

Finally, most of the groups of genes listed in Table 1 contain both "early" and "late" mutants. Therefore, none of the groups appears to be devoted to a single step of the retrotransposition cycle. Nevertheless, some of the groups have a disproportionate number of mutants devoted to either early or late stages of the retrotransposition cycle. For example, six of the seven transcription elongation mutants (*elp1*, *elp2*, *elp3*, *elp4*, *elp6*, and *kti12*) were found to affect the late stages of retrotransposition. All six of these "late" transcription elongation mutants, in principle, could affect retrotransposition by affecting the transcription of even a single "late" gene. Thus, our screen may have identified groups of genes involved in other processes (such as transcription elongation) that

are necessary for retrotransposition. This might help to account for the large number of mutants identified in our study. Additional secondary screens and assays will be necessary to identify these groups and to determine how such factors work together to influence retrotransposition.

Diminished tRNA gene targeting in *rit1* and *ctk1* mutants: Although most of the mutants identified in our study retained the ability to target Ty1 integration to tRNA genes, two of the mutants, *rit1* and *ctk1*, had diminished levels of tRNA gene targeting in our PCR assay (Figure 3). The Rit1 protein, which is an ADP ribosylase, is known to modify the methionine tRNA that serves as a primer for Ty1 strong-stop synthesis during cDNA replication (Chapman and Boeke 1991; Astrom and Bystrom 1994). Therefore, the *rit1* mutant might have been expected to affect cDNA replication. Although the *rit1* strain appeared to have slightly diminished levels of cDNA (Figure 4B), the average for the duplicate cDNA measurements was considered to be within the "normal" range (70.5% of wild type). An alternative model would be that *rit1* affects the efficiency of methionine tRNA cleavage from the end of the newly replicated cDNA (Lauermann and Boeke 1997). If the cDNA lacked the appropriate end structure as a result of faulty end trimming in a *rit1* mutant, it would not be expected to serve as a substrate for Ty1 integrase and may not be integrated efficiently into the genome. Similar cDNA end mutants have been shown to form multimers that are integrated into the genome by homologous recombination rather than by the normal integrase-mediated mechanism (Sharon *et al.* 1994). Thus, by interfering with cDNA end processing, *rit1* might promote a shift toward integration by homologous recombination.

We also observed a decrease in tRNA gene targeting in the *ctk1* mutant (Figure 3B). Ctk1p is a protein kinase known to regulate RNA polymerase II (RNA pol II) activity by phosphorylating the largest subunit of RNA polymerase II, Rpo21p (Patturajan *et al.* 1999). One possible explanation for the diminished targeting in this mutant would be that *ctk1* affects the RNA pol II transcription of a presently unknown host factor required for efficient targeting. Such factors might include proteins involved in RNA pol III transcription, for example. An alternative model would be that Ctk1p directly regulates RNA polymerase III activity. Since RNA pol III transcription, or an associated activity, is required for efficient tRNA gene targeting, altered phosphorylation of an RNA pol III subunit might be expected to have an impact on Ty1 integration.

A comparison of studies using Gal-Ty1 *vs.* chromosomal donor elements: Scholes *et al.* (2001) recently identified a large collection of Ty1 host mutants that had *increased* levels of Ty1 retrotransposition compared to wild-type strains. We found little overlap between those Ty1 host mutants and the host factors identified in our screen. The most likely explanation for this result is that Scholes *et al.* (2001) screened for mutants with *increased* levels of retrotransposition using a chromosomal Ty1 donor element, whereas we screened for mutants with *decreased* levels of retrotransposition using a Gal-Ty1 donor plasmid. Decreases might be difficult to detect at the already low levels of retrotransposition attained with the chromosomal assay, whereas further increases may not be easily achieved at the relatively high levels of retrotransposition produced with a Gal-Ty1 donor plasmid assay. There also were several other technical differences between these two studies. For example, the chromosomal donor assay used in the Scholes *et al.* (2001) study requires an mRNA splicing event to detect retrotransposition, whereas our Gal-Ty1 assay does not. Also, a fraction of the events that are recovered with a chromosomal donor assay occur by homologous recombination between the newly replicated cDNA and the original donor element. This problem is avoided with a Gal-Ty1 donor plasmid, because the donor plasmid is eliminated during the course of the assay on medium containing 5-FOA (and thus any events that target the donor plasmid by homologous recombination are eliminated from the final analysis). Finally, the strains used in these two studies were different. The mutants in the Scholes *et al.* (2001) study were constructed in a *MAT*α haploid strain background, whereas the mutants in our genomic screen were constructed in a diploid strain background.

Other studies also suggest that these experimental variables have an impact on retrotransposition experiments. For example, two previous studies reported seemingly contradictory results on the effects of *rad52* mutations on Ty1 retrotransposition (Sharon *et al.* 1994; Rattray *et al.* 2000). In the first study, a *rad52* deletion caused a relatively minor (up to a 2.7-fold) decrease in Ty1 retrotransposition (Sharon *et al.* 1994), whereas in the second study, a *rad52* mutation led to a 24-fold *increase* in Ty1 retrotransposition (Rattray *et al.* 2000). Although the basis for these contradictory results is unclear, it is likely that the differences are due, at least in part, to differences in the assays or strains used in the two studies. A Gal-Ty1 plasmid was used to measure Ty1 retrotransposition in the Sharon *et al.* (1994) study, whereas a chromosomal donor element with an artificial intron was used to measure retrotransposition in the Rattray *et al.* (2000) study. The strains used in the two studies also had some differences. For example, the strains in the Sharon *et al.* (1994) study contained an *spt3* mutation, whereas the strains in the Rattray *et al.* (2000) study did not. Nevertheless, even though some of the results obtained in these studies are apparently contradictory, these systems may together reproduce retrotransposition activities that occur naturally under different cellular conditions. Thus, all of these studies are likely to be informative.

We found that a *rad52* mutation led to a modest decrease in Ty1 retrotransposition, in agreement with

the Sharon *et al.* (1994) study outlined above. We observed a 4-fold decrease in retrotransposition, whereas Sharon *et al.* (1994) reported up to a 2.7-fold decrease in retrotransposition. Therefore, our results were similar to those obtained in the Sharon *et al.* (1994) study, in which a Gal-Ty1 plasmid also was used to measure retrotransposition. We also observed a 4-fold decrease in retrotransposition in an *xrs2* deletion strain, whereas Scholes *et al.* (2001) reported a 3- to 4-fold *increase* in retrotransposition in an *xrs2* mutant. As discussed above, these differences are likely to be caused by the different assays and strains used in these studies.

Additional comparisons with previous Ty1 host factor screens: A number of additional host factors have been identified that affect the Ty1 retrotransposition cycle (Winston *et al.* 1984; Boeke and Sandmeyer 1991; Chapman and Boeke 1991; Rinckel and Garfinkel 1996; Qian *et al.* 1998; Curcio and Garfinkel 1999; Huang *et al.* 1999; Bolton *et al.* 2002; and references therein). Upon comparing our genome-wide screen with these previous studies, we found that most of the factors identified in our screen were novel. Because our study was limited to the homozygous diploid deletion collection, we did not detect any host factors that were encoded by essential genes. We also did not generally detect *spt* mutants, because we used a *GAL1* promoter instead of the normal LTR promoter to circumvent most of the *spt* mutants. Nevertheless, we did detect four *spt* mutants, *spt2*, *spt4*, *spt10*, and *spt21*, and all four of these had altered levels of Ty1 cDNA. Because these mutants did not affect the *GAL1* promoter used on our Gal-Ty1 plasmid, these *spt* mutants must affect one of the remaining early steps of the retrotransposition cycle leading up to the assembly of virus-like particles and cDNA replication.

As expected, we identified the *dbr1* gene in our screen and observed a decrease in retrotransposition that was similar to the decrease reported previously (Chapman and Boeke 1991). We also identified the *pmr1* gene in our screen (Bolton *et al.* 2002). Pmr1 is a calcium-transporting ATPase that has been shown to influence the production of Ty1 cDNA (Bolton *et al.* 2002). However, *pmr1* was set aside in our study because it did not grow well on YPG medium containing glycerol as the sole carbon source. We used YPG medium as a secondary screen to avoid mutants that could not support respiration and thus might not utilize galactose efficiently in our retrotransposition assay. A total of 86 strains were set aside for this reason, although only a small fraction also had retrotransposition phenotypes. In the case of *pmr1*, it appears that this secondary screen was too stringent and led to the elimination of a true positive (Bolton *et al.* 2002). However, in most cases, problematic strains were set aside with this secondary screen, and such strains often grew poorly on at least one additional growth medium.

Potential Ty1 host factor homologs in other organisms: Potential Ty1 host factor homologs were identified in a number of other organisms by performing BLAST searches of the public databases with the host factors identifed in our study (Altschul *et al.* 1990). Full-length ORF translations for each of the genes listed in Table 1 were obtained from the *Saccharomyces* Genome Database (http://genomewww.stanford.edu/Saccharomyces/). These sequences then were used as BLAST queries against the nonredundant protein database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) using the default settings. Potential homologs were identified in a variety of organisms, including humans, with this approach. Using a BLAST Expect value cutoff of $<10^{-13}$, 39 of the 101 genes listed in Table 1 encoded proteins with significant matches to potential human homologs (data not shown). Even with a more stringent BLAST Expect value cutoff of $<10^{-30}$, 27 of the queries had significant matches to potential human homologs. Similar results were obtained for mouse and other organisms (data not shown). Thus, our study can serve as a starting point for identifying and studying host factors that affect LTR retroelements and retroviruses in other organisms. Such factors could be useful in humans, for example, as intracellular drug targets for the treatment of AIDS.

In conclusion, our study has increased the number of host factors known to affect Ty1 and, together with previous studies, provides a more complete picture of the relationship between Ty1 and its yeast host. Our study provides a foundation for many future studies on the retrotransposition cycle. Functional genomics screens such as ours, using the complete yeast knockout collection, will be very useful for identifying the nonessential genes involved in a given cellular process and will be invaluable for dissecting complex biological processes.

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