Multiple Regulators of Ty1 Transposition in *Saccharomyces cerevisiae* Have Conserved Roles in Genome Maintenance

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ABSTRACT

Most Ty1 retrotransposons in the genome of *Saccharomyces cerevisiae* are transpositionally competent but rarely transpose. We screened yeast mutagenized by insertion of the mTn3-*lacZ/LEU2* transposon for mutations that result in elevated Ty1 cDNA-mediated mobility, which occurs by cDNA integration or recombination. Here, we describe the characterization of mTn3 insertions in 21 *RTT* (*r*egulation of *T*y1 *t*ransposition) genes that result in 5- to 111-fold increases in Ty1 mobility. These 21 *RTT* genes are *EST2*, *RRM3*, *NUT2*, *RAD57*, *RRD2*, *RAD50*, *SGS1*, *TEL1*, *SAE2*, *MED1*, *MRE11*, *SCH9*, *KAP122*, and 8 previously uncharacterized genes. Disruption of *RTT* genes did not significantly increase Ty1 RNA levels but did enhance Ty1 cDNA levels, suggesting that most *RTT* gene products act at a step after mRNA accumulation but before cDNA integration. The *rtt* mutations had widely varying effects on integration of Ty1 at preferred target sites. Mutations in *RTT101* and *NUT2* dramatically stimulated Ty1 integration upstream of tRNA genes. In contrast, a mutation in *RRM3* increased Ty1 mobility >100-fold without increasing integration upstream of tRNA genes. The regulation of Ty1 transposition by components of fundamental pathways required for genome maintenance suggests that Ty1 and yeast have coevolved to link transpositional dormancy to the integrity of the genome.

LONG terminal repeat (LTR) retrotransposons are eukaryotic mobile elements that resemble retroviral proviruses and transpose through an RNA intermediate. Integration of LTR retrotransposons into genomic DNA is a potential source of mutagenesis to the host cell. A selective advantage is therefore conferred upon a host that has evolved mechanisms to reduce the level of retrotransposition or its mutagenic effects. Ty1 retrotransposons in yeast exhibit transpositional dormancy, characterized by the collective inactivity of genetically functional elements. The majority of the ~30 Ty1 elements in the haploid yeast genome is free of inactivating mutations and competent for transposition (Curcio and Garfinkel 1994; Jordan and McDonald 1998; Kim *et al.* 1998). Moreover, Ty1 RNA is one of the most abundant mRNA species in yeast, contributing up to 0.8% of total RNA (Curcio *et al.* 1990). Despite this, transposition occurs at a rate of only 10^{-5}–10^{-7}/element/generation (Curcio and Garfinkel 1991). Cytoplasmic virus-like particles (VLPs), in which Ty1 RNA is reverse-transcribed into cDNA, are difficult to detect in most laboratory strains, and there is less than one copy of Ty1 cDNA per cell (Conte *et al.* 1998; Lee *et al.* 1998). These findings suggest that transpositional dormancy results from inhibition of one or more post-transcriptional steps in the Ty1 replication cycle. No intrinsic mechanisms of regulating Ty1 transposition have yet been described; however, host factors that inhibit Ty1 transposition at different post-transcriptional levels have been identified (Picologlou *et al.* 1990; Conte *et al.* 1998; Lee *et al.* 1998; Rattray *et al.* 2000; Bryk *et al.* 2001).

The low levels of VLPs in normal yeast cells suggest that transposition may be regulated at the level of translation, protein processing, or protein stability. Regulation of Ty1 mRNA translation has not been described, but it is known that proteolytic processing of Ty1 proteins is extremely inefficient (Curcio and Garfinkel 1992). Instability of Ty1 proteins is regulated by the mitogen-activated protein kinase, Fus3, which inhibits Ty1 transposition 18- to 56-fold by stimulating the degradation of VLP-associated Ty1 proteins (Conte *et al.* 1998). Fus3 regulates Ty1 transposition by negatively regulating the invasive growth pathway, which activates Ty1 transposition at both transcriptional and post-translational levels (Conte and Curcio 2000; Morillon *et al.* 2000).

The characterization of two additional inhibitors of Ty1 transposition has shown that cDNA degradation is a critical step in the maintenance of transpositional dormancy. Certain mutations in *SSL2* and *RAD3*, which encode components of the RNA Polymerase II general transcription factor, TFIIH, increase Ty1 transposition 100-fold or more (Lee *et al.* 1998). Moreover, unintegrated Ty1 cDNA is stabilized in *ssl2-rtt* and *rad3-G595R* mutants (Lee *et al.* 2000). Several other inhibitors of Ty1 transposition may act by promoting cDNA degradation,

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including members of the *RAD52* recombinational repair pathway (*RAD50*, *RAD51*, *RAD52*, *RAD54*, and *RAD57*) and *CDC9*, which encodes DNA ligase (Rattray *et al.* 2000).

We recently demonstrated that the RecQ-helicase, Sgs1, limits the mobility of Ty1 elements by altering the fate of Ty1 cDNA (Bryk *et al.* 2001). Although Ty1 cDNA levels are modestly elevated in *sgs1*Δ mutants, accumulation of cDNA is not the major cause of increased Ty1 mobility. Instead, recombination between extrachromosomal cDNA molecules is stimulated in *sgs1*Δ mutants, resulting in formation of multimeric Ty1 cDNA arrays that integrate into the genome. These findings indicate that cDNA can be directed into different pathways of integration, degradation, or recombination and suggest that the processing of Ty1 cDNA may be strongly influenced by host genes.

Inaccessibility of integration targets may also contribute to transpositional dormancy. Ty1 elements integrate primarily into regions upstream of RNA Pol III-transcribed genes or, more rarely, into the promoter regions of RNA Pol II-transcribed genes, but open reading frames (ORFs) are poor targets for integration (Ji *et al.* 1993; Devine and Boeke 1996). Mutations in host genes that increase transposition into Pol II-transcribed ORFs have been identified (Picologlou *et al.* 1990; Liebman and Newnam 1993; Qian *et al.* 1998; Huang *et al.* 1999). These include mutations in *RAD6*, which encodes a ubiquitin-conjugating protein, and concurrent mutations in *CAC3*, which encodes a subunit of chromatin assembly factor-1, and *HIR3*, which encodes a regulator of histone gene transcription. Simultaneous inactivation of *CAC3* and *HIR3* also resulted in a three- to fivefold increase in the mobility of a chromosomal Ty1 element, suggesting that *CAC3* and *HIR3* may limit the accessibility of target sites for Ty1 integration (Qian *et al.* 1998).

Most of the characterized regulators of Ty1 transposition described above were identified on the basis of their effect on the mobility of a Ty1 element marked with the retrotranscript indicator gene *his3AI* (Conte *et al.* 1998; Lee *et al.* 1998; Rattray *et al.* 2000; Bryk *et al.* 2001). The cDNA-mediated mobility of a Ty1*his3AI* element can be quantified in a simple phenotypic assay, regardless of the target of cDNA integration or recombination (Figure 1). Thus, this approach can facilitate the identification of mutations that affect different steps of Ty1 transposition. Chemical mutagenesis in strains containing Ty1*his3AI* elements has previously been attempted to identify regulators of transpositional dormancy (Conte *et al.* 1998; Lee *et al.* 1998; M. Bryk and M. J. Curcio, unpublished results). While these screens yielded large numbers of Rtt$^-$ mutants, none of the underlying mutations have been successfully identified by complementation. In this study, we used transposon-mediated mutagenesis (Burns *et al.* 1994) to circumvent the problems associated with cloning by complementation. Chromosomal mutations tagged with a mTn3-*lacZ/LEU2* transposon were generated, allowing rapid recovery of mutations and identification of the affected gene. Using this method, we characterized 21 genes that encode *r*egulators of *T*y1 *t*ransposition (*RTT* genes), 18 of which have not been previously shown to affect transposition. Most or all of the *RTT* gene products inhibit post-transcriptional steps in transposition, and most have a discernible effect on unintegrated Ty1 cDNA levels. Many *RTT* gene products have roles in genome maintenance, including telomere maintenance, DNA recombinational repair, suppression of DNA recombination, and DNA-damage response pathways. The finding that Ty1 transposition is regulated by a large number of proteins involved in DNA metabolism suggests that Ty1 transposition levels are modulated in response to changes in the integrity of the genome.

MATERIALS AND METHODS

Yeast strains and media: Standard yeast culture media were prepared as described (Rose and Broach 1990). The following yeast strains are all derivatives of strain GRF167 (Boeke *et al.* 1985). Strain JC2326 [*MAT*Δ-*ura3*, cir^0, *ura3*-167, *leu2::hisG*, *his3*Δ200, Ty1*his3AI*-270, Ty1*NEO*-588, Ty1(*tyb1::lacZ*)-146] and strain JC2749 [*MAT*α, *trp1::hisG*, cir^0, *ura3*-167, *leu2::hisG*, *his3*Δ200, Ty1*his3AI*-270, Ty1*NEO*-588, Ty1(*tyb1::lacZ*)-146] were constructed from strain JC344 [*MAT*α, *ura3*-167, *leu2::hisG*, *his3*Δ200, Ty1*his3AI*-270, Ty1*NEO*-588, Ty1(*tyb1:: lacZ*)-146; Kawakami *et al.* 1993] as follows. Strain JC344 was cured of the endogenous 2μ plasmid (cir^0) by introducing plasmid YEp351-*GAL-FLP1* (Rose and Broach 1990) into the strain and overexpressing the *FLP1* gene by growth in medium containing galactose. The *MAT*α information was subsequently deleted by one-step transplacement of a fragment containing the *MAT*Δ-*URA3* allele [a plasmid carrying this fragment was a gift from J. Strathern, National Cancer Institute (NCI)-Frederick Cancer Research and Development Center (FCRD)]. A spontaneous Ura$^-$ derivative of this strain was obtained by selection on 5-fluoroorotic acid (5-FOA) medium to construct strain JC2326. Strain JC2749 was constructed by transforming the cir^0 derivative of strain JC344 with the *trp1::hisG-URA3-hisG* allele (Alani *et al.* 1987), followed by selection for a *trp1::hisG* derivative on 5-FOA medium. Strain JC270 [*MAT*α, *ura3*-167, *his3*Δ200, Ty1*his3AI*-270, Ty1*NEO*-588, Ty1(*tyb1::lacZ*)-146] is an isogenic *LEU2* derivative of strain JC344. Strain JC357 [*MAT***a**-*URA3*, *leu2::hisG*, *ade2*, *his3*Δ200, Ty1*NEO*-588, Ty1*his 3*AI-270], which contains the *URA3* gene integrated between the *MAT***a** and *CRY1* loci, is an ascospore derived by crossing strain JC344 with strain GRY340 (Curcio *et al.* 1988) and then backcrossing a selected ascospore to strain JC344 twice. Strains JC2148 (*MAT*α, *ura3*-167, *his3*Δ200, *leu2::hisG*, *tec1*Δ*::ura3* Ty1*his3AI*-270; Conte and Curcio 2000) and DG789 (*MAT*α, *his3*Δ*200*, *ura3-167*, *spt3-101*; Curcio and Garfinkel 1991) were described previously. Strain DG1722 (*MAT*α, *ura3*-167, *his3*Δ200, *ssl2-rtt*) is described in Lee *et al.* (1998) and was generously provided by D. Garfinkel (NCI-FCRDC). Strain JC384 (*MAT*α, *his3*Δ*200*, *ura3-167 trp1::hisG*) is a *trp1::hisG* derivative of GRF167 harboring plasmid pGTy1-H3m*HIS3* (Curcio and Garfinkel 1991).

Strain BY4742 (*MAT*α, *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0; Brachmann *et al.* 1998) and derivatives, each containing the precise replacement of a specific ORF with the *kanMX4* module (Winzeler *et al.* 1999), were obtained from Research Genetics (Birmingham, AL). A *tlc1*Δ*::LEU2* derivative of BY4742 was

constructed by one-step transplacement using pBLUE61::*LEU2* (Singer and Gottschling 1994). A Ty1*his3AI[Δ1]-URA3* cassette was introduced into strain BY4742 and isogenic ORF deletion strains by transformation of plasmid pJC573 linearized with *Pac*I. Strains in which plasmid pJC573 is integrated are JC3116 (BY4742), JC3118 (BY4742, *rtt110Δ::kanMX4*), JC3122 (BY4742, *rmr1Δ::kanMX4*), JC3134 (BY4742, *rtt107Δ::kanMX4*), JC3138 (BY4742, *rrm3Δ::kanMX4*), JC3142 (BY4742, *mre11Δ::kanMX4*), JC3144, JC3493 (both BY4742, *tel1Δ::kanMX4*), JC3198 (BY4742, *rtt101Δ::kanMX4*), JC3199 (BY4742, *rtt109Δ::kanMX4*), JC3200 (BY4742, *kap122Δ::kanMX4*), JC3497 (*est1Δ::kanMX4*), JC3503 (*est2Δ::kanMX4*), JC3519 (*rif1Δ::kanMX4*), JC3520 (*rif2Δ::kanMX4*), JC3368 (*xrs2Δ::kanMX4*), and JC3489 (BY4742, *tlc1Δ::LEU2*).

Plasmids: Plasmid pJC573 contains 1.2 kb of yeast genomic DNA from the *BIK1-HIS4* intergenic region on chromosome III adjacent to a Ty1 element in the *URA3*-based integrating vector pRS406 (Sikorski and Hieter 1989). The modified retrotranscript indicator gene, *his3AI[Δ1]*, was cloned into the Ty1 element at the *Bgl*II site in *TYB1*, adjacent to the 3′ LTR. The *his3AI[Δ1]* gene contains the same 104-bp artificial intron (AI) as *his3AI* (Curcio and Garfinkel 1991) inserted at a different position (+440) in the *HIS3* ORF. At this location, the AI is within the interval that is deleted in the *his3Δ1* allele in strain BY4742, thereby eliminating the formation of a functional *HIS3* gene by DNA recombination. Construction of plasmid pJC573 is described elsewhere (Bryk *et al.* 2001). Plasmid pJC525 contains a 934-bp *Hin*dIII-*Bgl*II fragment of Ty1-H3 (nucleotides 4627–5561; Boeke *et al.* 1986) cloned into plasmid vector pSP70 (Promega, Madison, WI).

Mutagenesis screen: A yeast genomic DNA library containing random insertions of the bacterial transposon mTn3-*lacZ/LEU2* (Burns *et al.* 1994) was generously provided by M. Snyder (Yale University). Strains JC2326 and JC2749 were transformed with ~1 μg of library DNA digested with *Not*I. Leu^+ transformants (50 per plate) and the *LEU2* strain JC270 were grown in small patches on SC-Leu plates at 30° for 2 days. Subsequently, mTn3-*lacZ/LEU2* transformants were replicated to YPD plates, grown at 20° for 3 days, and then replicated to SC-His medium and grown at 30° for 3 days. Patches of transformants with at least four His^+ papillae were selected for further analysis. (Strain JC270 had 0 or 1 His^+ papillae per patch.) Selected mTn3-*lacZ/LEU2* transformants were single-colony purified on SC-Leu medium. Large patches of each Leu^+ strain (12 per plate) and the isogenic wild-type strain (JC2326 or JC2749) were grown on YPD medium at 30°, replicated to YPD medium and grown at 20° for 3 days, and then replicated to SC-His and grown at 30° for 3 days. Transformants with elevated levels of His^+ papillation (Rtt^- phenotype) relative to JC2326 or JC2749 were saved for further analysis. Following the identification of the mTn3-*lacZ/LEU2* insertion site in 112 Rtt^- mutants (see below), a second screen for elevated His^+ prototroph formation was performed by streaking each mutant and strain JC2326 or JC2749 for single colonies on one-quarter of a YPD plate and incubating at 20° for 6 days. Colonies were replicated to SC-His medium and grown for 3 days at 30° before scoring His^+ prototrophs.

Identification of the mTn3-*lacZ/LEU2* insertion sites: Genomic DNA from *rtt::*mTn3-*lacZ/LEU2* mutant strains was prepared from a saturated 10-ml YPD culture using the G'NOME kit (BIO 101, Vista, CA) and resuspended in 100 μl TE. DNA (15 μl) was digested with 10 units *Rsa*I in a total volume of 100 μl, diluted 1:10, and ligated with 100 units T4 ligase. Using oligomers InPCR1 (5′-TAAGTTGGGTAACGCCAGGG TTTTC-3′) and InPCR2 (5′-TTCCATGTTGCCACTCGCTT TAATG-3′), the 5′ junction of mTn3-*lacZ/LEU2* with genomic DNA was amplified. A 213-bp fragment of the Ty1(*tyb1::lacZ*)-146 allele in each strain was amplified by the same primers. The products of each PCR reaction were analyzed by agarose gel electrophoresis. An aliquot of each PCR reaction that yielded the 213-bp control band was subject to DNA sequencing on an ABI sequencer using the oligomer mTn3-SEQ (5′-CCCCCTTAACGTGAGTTTTCGTTCCACT-3′).

Tetrad analysis: To perform tetrad analysis, the mating type of *MAT*Δ strains was changed to *MAT*α by two-step gene disruption using plasmid pSC9, a *URA3*-based integrating vector harboring the *MAT*α allele (Adams *et al.* 1997). Alternatively, the *rtt::*mTn3-*lacZ/LEU2* alleles in the *MAT*Δ*::URA3* strain JC2326 were transferred to the *MAT*α strain JC2749 by "whole genome transformation." Approximately 50 μg of genomic DNA prepared from *rtt::*mTn3 mutants as described in Conte *et al.* (1998) was transformed into strain JC2749 without carrier DNA, and Leu^+ transformants were selected. Following single colony purification, Leu^+ transformants with a hypermobility phenotype similar to that of the corresponding *MAT*Δ strain were isolated. Southern analysis with a *LEU2* probe was performed to confirm the presence of the *rtt::*mTn3 disruption allele. *MAT*α *rtt::*mTn3 strains were crossed with *MAT***a** strain JC357. Sporulation of the resulting diploids was induced, and tetrads were dissected by standard methods (Ausubel *et al.* 1993). The level of His^+ prototroph formation in each spore was determined by growing each spore as a patch on YPD plates at 30°, replicating to YPD and growing at 20° for 3 days, and then replicating to SC-His and growing for 3 days at 30°.

Ty1 cDNA-mediated mobility assays: The frequency of His^+ prototroph formation in strains containing the chromosomal Ty1*his3AI*-270 or Ty1*his3AI[Δ1]* element was determined as follows. Cultures of each yeast strain in 5 ml YPD broth were grown to saturation at 30°. Each culture was diluted 1:1000 in 2 ml YPD medium and grown to saturation at 20°. The number of cells per culture was determined by plating 0.002 μl on YPD medium (strains JC2326, JC2749, and derivatives) or SC-Ura medium (strain JC3116 and derivatives). A 400-μl aliquot of cultures of strains JC2326 and JC2749 and a 100-μl aliquot of cultures of each *rtt::*mTn3 derivative were plated on SC-His medium. A 400-μl aliquot of cultures of strain JC3116 and a 100-μl aliquot of cultures of each *rtt*Δ derivative were spread on SC-Ura-His medium. The transposition frequency is the average number of His^+ prototrophs per cell from three or four independent cultures (strains JC2326, JC2749, and derivatives) or of His^+ Ura^+ prototrophs per Ura^+ cell from four, five, or six cultures (strain JC3116 and derivatives).

To determine the rate of His^+ prototroph formation, 5-ml cultures of each strain were grown to saturation at 30° in liquid YPD medium. Eleven tubes containing 2 ml YPD medium were inoculated with 2 μl of the saturated culture and grown at 20° to saturation. A 100-μl aliquot of *rtt* mutant cultures and 400-μl aliquots of strain JC2326 and JC2749 cultures were plated on SC-His medium. The titer of four cultures of each strain was determined by plating 0.002 μl on YPD medium. The rate of His^+ prototroph formation per cell per generation was evaluated by the method of Lea and Coulson (1949).

Northern analysis: By hot acidic phenol extraction (Ausubel *et al.* 1993), total RNA was isolated from 50-ml cultures of each strain grown in YPD broth at 20° to midexponential phase (OD_{600} = 1.0). RNA samples denatured with glyoxal were subject to electrophoresis in a 1% agarose gel and transferred to a Hybond-N membrane (Amersham, Arlington Heights, IL). Ty1*his3AI*, Ty1, and *PYK1* transcripts were detected using ^{32}P-labeled *HIS3* sense-strand, Ty1 antisense-strand, and *PYK1* antisense-strand riboprobes, respectively. Riboprobes were synthesized using plasmid pGEM-HIS3, plasmid pGEM-TyA1 (Curcio *et al.* 1990), or plasmid pGEM-PYK1 (Curcio and Garfinkel 1992) as template DNA. Northern blot banding patterns were visualized by autoradiography. The

^{32}P activity in each band was quantitated using a STORM 860 phosphorimager and ImageQuant software.

cDNA analysis: Single colonies of each strain grown at 20° were used to inoculate cultures of 15 ml YPD broth, and two or three cultures were grown at 20° to stationary phase. Total yeast genomic DNA was extracted as described in Conte *et al.* (1998) and digested with *Pvu*II. DNA samples were subject to electrophoresis on a 1% agarose gel and transferred to a Hybond-N+ membrane (Amersham). The membrane was hybridized to a ^{32}P-labeled *TYB1* antisense riboprobe prepared using plasmid pJC525 as a template. Southern blot bands were visualized by autoradiography, and the ^{32}P activity was quantitated using a STORM 860 phosphorimager and ImageQuant software.

Integration assay: Single colonies grown at 20° were used to inoculate cultures of 15 ml of YPD broth, which were grown at 20° to stationary phase. Total genomic DNA was extracted as described in Ausubel *et al.* (1993). To confirm that the genomic DNA samples were equivalently competent for PCR, fragments of single copy genes were amplified from genomic DNA, separated by agarose gel electrophoresis, and quantitated by ethidium staining. To detect Ty1 integration events at glycyl-tRNA genes, oligonucleotides TYBOUT-2 (5′-GTGA TGACAAAACCTCTTCCG-3′) and SUF16-2 (5′-GGCAACGT TGGATTTTACCAC-3′) were used in 50-μl PCR reactions using the Failsafe PCR kit (Epicentre Technologies, Madison, WI). Reactions contained 1× PreMix E, 0.4 μM oligonucleotide TYBOUT-2, 0.4 μM oligonucleotide SUF16-2, 1.25 units Failsafe enzyme mix, and 0.1 μg genomic DNA. Cycling conditions were 94° for 2 min; followed by 10 × (94° for 30 sec, 65° for 30 sec, 72° for 60 sec); followed by 20 × (94° for 30 sec, 60° for 30 sec, 72° for 60 sec); followed by 72° for 5 min; followed by cooling to 4°. PCR products were run on a 2% agarose gel and transferred on to a Hybond-N+ membrane. The membrane was probed with the oligonucleotide SUF16-START (5′-GGATTTTACCACTAAACCACTTGCGC-3′) end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Southern blot bands were visualized by autoradiography.

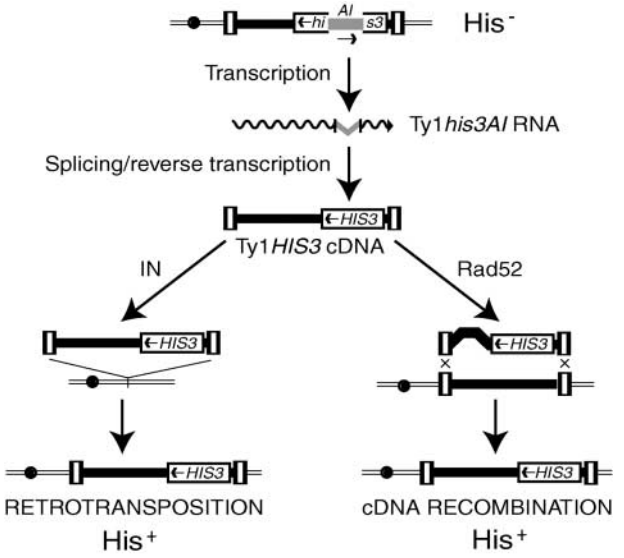

Figure 1.—Assay for Ty1 cDNA-mediated mobility. A genomic Ty1 element is represented by LTRs (tripartite rectangles) surrounding a coding region (solid bar) within chromosomal DNA (two thin lines with a circle representing the centromere). The *HIS3* gene (labeled box) has been introduced into the Ty1 element, with its coding sequence in the opposite orientation (indicated by arrow) to that of Ty1. The *HIS3* gene is rendered nonfunctional by the presence of an artificial intron (AI; shaded bar) in the opposite orientation (indicated by arrow) to that of the *HIS3* gene. AI is not recognized as an intron in the *HIS3* transcript and therefore cannot be spliced out. However, AI is spliced out of the Ty1*his3*AI transcript (wavy line with spliced AI indicated by shaded bars between two vertical solid bars). Subsequent reverse transcription of the spliced Ty1 transcript generates a Ty1 cDNA containing a functional *HIS3* gene. The Ty1*HIS3* cDNA can enter the genome by integration of Ty1*HIS3* cDNA into a *de novo* site, mediated by IN (arrow on left), or by recombination of the Ty1*HIS3* cDNA with a genomic Ty1 element, mediated by Rad52 (arrow on right). Both pathways result in formation of a His$^+$ prototroph.

RESULTS

A genetic screen identifies 29 regulators of Ty1 transposition: To identify novel genes involved in the maintenance of Ty1 transpositional dormancy, we performed a screen for host mutations that result in increased mobility of a chromosomal Ty1 element marked with *his3AI* (Curcio and Garfinkel 1991). The mobility of Ty1*his3AI* elements is detected phenotypically by the formation of His$^+$ prototrophs (Figure 1). His$^+$ colonies are indicative of cells that have sustained either nonhomologous integration of Ty1*HIS3* cDNA into the genome or homologous recombination of Ty1*HIS3* cDNA with preexisting genomic Ty1 elements or LTRs. Mutations in genes involved in the maintenance of Ty1 transpositional dormancy are expected to increase the formation of His$^+$ prototrophs, which is referred to as a hypermobility or Rtt$^-$ phenotype.

Transposon-mediated mutagenesis was performed by introducing a library of yeast genomic DNA fragments disrupted with mTn3-*lacZ/LEU2* into congenic yeast strains JC2326 (*MAT*Δ) and JC2749 (*MAT*α). Approximately 10,000 Leu$^+$ transformants were tested to determine their relative levels of His$^+$ prototroph formation. A total of 274 (2.7%) Leu$^+$ transformants had elevated levels of His$^+$ papillation relative to a congenic wild-type strain (Figure 2). Genomic DNA was isolated from 85 of the Rtt$^-$ strains and analyzed by Southern blotting with a *LEU2* probe (data not shown). Eighty-two of the Rtt$^-$ strains harbored a single mTn3-*lacZ/LEU2* insertion at a random location, whereas the other 3 strains had two mTn3-*lacZ/LEU2* insertions. Because almost all of the putative Rtt$^-$ mutants sustained only one insertion, we determined the location of the mTn3-*lacZ/LEU2* insertion by PCR amplification and sequencing of the junction between the 5′ end of the mTn3-*lacZ/LEU2* element and yeast genomic DNA. Thirty or more nucleotides of DNA sequence were obtained from 112 putative hypermobility mutants and compared to the sequence of the Saccharomyces genome. This analysis identified mTn3-*lacZ/LEU2* insertion sites within or upstream of 77 different annotated ORFs (Figure 2). The

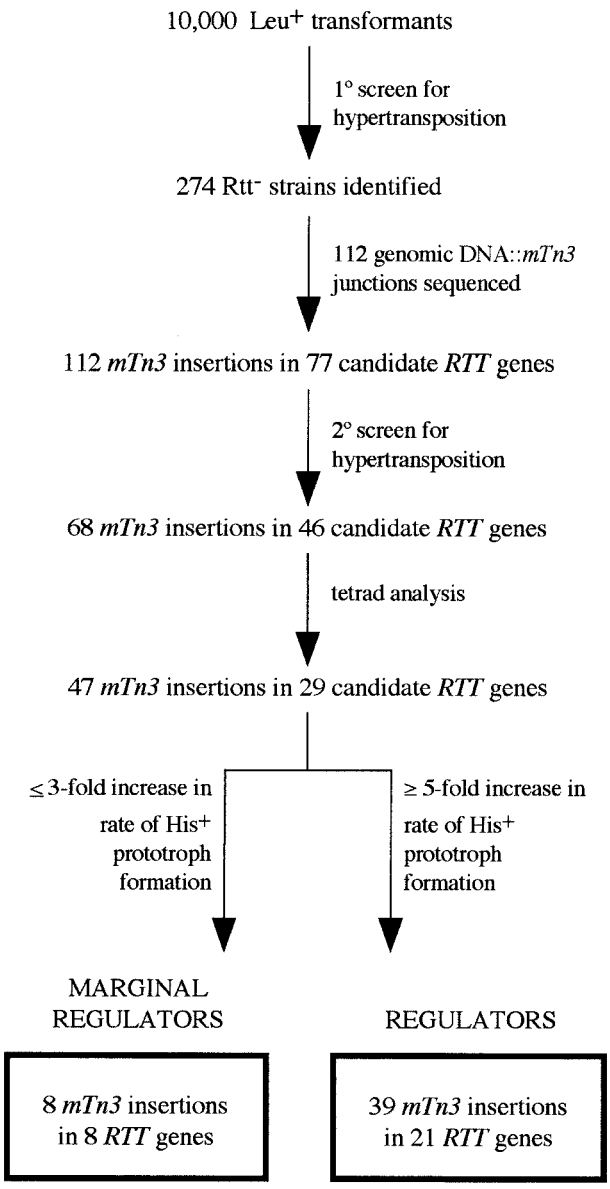

FIGURE 2.—Identification of *RTT* genes. The flow chart shows the methodology of the genetic screen for hypermobility mutants and the identification of *RTT* genes.

other 162 Rtt$^-$ mutants were not analyzed (122 strains), harbored multiple mTn3-*lacZ/LEU2* inserts (3 strains), or failed to yield useful inverse PCR products or DNA sequence (37 strains).

Each of the 112 candidate *rtt::*mTn3-*lacZ/LEU2* mutants was subject to a second qualitative test for His$^+$ prototroph. Sixty-eight putative *rtt* mutants had a consistently elevated level of His$^+$ prototroph formation. These 68 *rtt* mutants harbored mTn3-*lacZ/LEU2* elements in or upstream of 46 different annotated ORFs (Figure 2). The Rtt$^-$ phenotype of *MAT*Δ/α diploids heterozygous for each of the 68 *rtt* mutations was tested, revealing that all 68 mutations were recessive.

Tetrad analysis was performed on at least one mTn3-disruption allele of each of the 46 candidate *RTT* genes to confirm that the hypermobility phenotype was a result of the mTn3 insertion. One exception was mTn3 insertions in *SGS1*, which were not analyzed here because an *sgs1*Δ allele has already been shown to cosegregate with a hypermobility phenotype in tetrad analysis (BRYK *et al.* 2001). *MAT*α derivatives of *MAT*Δ *rtt::*mTn3-*lacZ/LEU2* strains were constructed, and then 49 *MAT*α *rtt::*mTn3-*lacZ/LEU2* strains were crossed to a congenic wild-type strain. Three diploids failed to yield tetrads with four viable spores. Tetrads from the other 46 diploids displayed 2:2 segregation of the Leu$^+$ phenotype, confirming the presence of a single mTn3-*lacZ/LEU2* insertion. One of the 46 strains showed independent segregation of the Rtt$^-$ and Leu$^+$ phenotypes, indicating that hypermobility was not caused by the mTn3-*lacZ/LEU2* disruption. Of the 45 remaining strains, 15 failed to show consistent 2:2 segregation of the Rtt$^-$ phenotype in tetrad analysis. These 15 *rtt::*mTn3-*lacZ/LEU2* candidates included one mTn3-disruption allele of 12 different putative *RTT* genes and three independent mTn3-disruption alleles of *YKU80*, which encodes the 80-kD subunit of Ku. Mutations in *YKU80* have previously been demonstrated to cause a small increase in the mobility of a Ty1*his3AI* element (DOWNS and JACKSON 1999). Our data suggest that the effect of *YKU80* on Ty1 cDNA-mediated mobility is strongly influenced by the genetic background in which it is tested. It was concluded that the effect of these 15 *rtt::*mTn3-*lacZ/LEU2* alleles on Ty1 mobility was dependent on heterozygous alleles of other genes that segregated independently in tetrad analysis.

The remaining 30 candidate *rtt::*mTn3 strains tested by tetrad analysis showed an Rtt$^-$ phenotype that cosegregated with the Leu$^+$ phenotype. These 30 *rtt::*mTn3 alleles included mutations in 28 different *RTT* genes, demonstrating that these 28 *RTT* genes consistently inhibit the cDNA-mediated mobility of Ty1 elements. Including the previously characterized regulator of Ty1 transposition encoded by *SGS1*, a total of 29 different *RTT* genes were identified in the screen. Forty-seven *rtt* mutations, including the 30 that were tested by tetrad analysis and 17 additional mutations within one of the same 29 genes, were isolated in the screen (Figure 2). These 47 *rtt* mutants harbor independent mTn3 insertions in, or within 84 bp upstream of, one of the 29 *RTT* ORFs (Tables 1 and 2). All 29 *RTT* genes are represented by at least one allele in which mTn3 is in the ORF, except *NUT2* (Table 1) and *MCM6* (Table 2), which are both essential.

Eight *rtt* mutants have a marginal increase in Ty1 cDNA-mediated mobility: To characterize the 29 *RTT* genes identified, we quantified the increase in mobility of the Ty1*his3AI*-270 element in *rtt::*mTn3 mutants by measuring the rate of His$^+$ prototroph formation (Tables 1 and 2). The relative rate of Ty1 mobility in 29 *rtt* mutants is indicated in Figure 3. In cases in which the relative mobility rate was determined for two different

TABLE 1

Eight marginal regulators of Ty1 transposition

Disrupted *RTT* gene	Strain	Parental strain	Insertion site of mTn3[a] (size of ORF)	Tetrads analyzed	Rate of Ty1*his3AI* mobility (±SE)[b]	Frequency of Ty1*his3AI* mobility (±SD)[c]	Relative cDNA level[d]
—	JC2326	—	—	—	$1.2\ (\pm0.5) \times 10^{-7}$[e]	$3.3\ (\pm2.4) \times 10^{-7}$[f]	1
—	JC2749	—	—	—	$8.9\ (\pm3.4) \times 10^{-8}$[e]	$8.9\ (\pm3.0) \times 10^{-7}$[f]	1
VAC8	JC2471	JC2326	+275 (1736)	8	$5.3\ (\pm2.3) \times 10^{-7}$	$2.1\ (\pm0.6) \times 10^{-7}$	1.3
HSP78	JC2807	JC2749	+1879 (2435)	6	$2.1\ (\pm1.0) \times 10^{-7}$	$1.5\ (\pm1.6) \times 10^{-6}$	1.6
RTT102/YGR275W	JC2482	JC2326	+300 (560)	13	$2.4\ (\pm1.2) \times 10^{-7}$	$2.3\ (\pm0.2) \times 10^{-7}$	0.9
MLP2	JC2836	JC2749	+3791 (5039)	5	$1.4\ (\pm0.7) \times 10^{-7}$	$3.7\ (\pm1.2) \times 10^{-7}$	1.1
MCM6	JC2470	JC2326	−35 (3053)	10	$1.5\ (\pm0.5) \times 10^{-7}$	$1.0\ (\pm0.5) \times 10^{-6}$	1.8
TIF4632	JC2491	JC2326	+17 (2744)	15	$3.3\ (\pm1.4) \times 10^{-8}$	$1.1\ (\pm1.2) \times 10^{-6}$	1.1
RNR1	JC2509	JC2326	+895 (2666)	22	$1.3\ (\pm0.5) \times 10^{-7}$	$1.6\ (\pm0.1) \times 10^{-6}$	2.3
RFX1	JC2427	JC2326	+1029 (2435)	4	$2.5\ (\pm0.9) \times 10^{-7}$	$3.1\ (\pm1.0) \times 10^{-7}$	1.7

[a] Number of nucleotides from the beginning of the ORF (+1).
[b] Number of His^+ prototrophs per cell per generation ± standard error.
[c] Number of His^+ prototrophs per cell ± standard deviation.
[d] The amount of Ty1 cDNA relative to parental strain JC2326 or JC2749, as determined by quantitative Southern analysis.
[e] The transposition rate of wild-type strains JC2326 and JC2749 was evaluated in successive experiments. Presented here is the result obtained in which the variance was smallest.
[f] Average of values obtained in successive experiments.

mTn3 disruption alleles of the same *RTT* gene, the strain with the higher value is reported in Figure 3. In eight *rtt* mutants tested, the relative mobility rate was less than threefold higher than that of the isogenic wild-type strain (Figure 3). Hence, strains harboring mTn3 disruption alleles of eight different *RTT* genes, which are listed in Table 1, caused a minor or indiscernible increase in Ty1 mobility when assayed quantitatively, even though they displayed a consistently elevated level of His^+ prototroph formation in qualitative plate assays, even through tetrad analysis. This class of marginal *RTT* genes includes one essential gene, *MCM6*, and another gene that is essential in some strain backgrounds, *RNR1* (Table 3). The mTn3 insertion is 35 bp upstream of the *MCM6* ORF (Table 1), suggesting that it may affect but not abolish the level of *MCM6* expression. In contrast, the mTn3 insertion in *RNR1* is at nucleotide 895 of the 2666-nucleotide ORF, and therefore it may be a null mutation. The six other *RTT* genes in this class include *RTT102*, which was identified as the uncharacterized ORF *YGR275W*, as well as *VAC8*, *HSP78*, *MLP2*, *TIF4632*, and *RFX1* (Tables 1 and 3).

The mTn3-*lacZ/LEU2* transposon is located within the ORF of seven of these eight *RTT* genes (Table 1), suggesting that the *rtt::*mTn3 alleles are null alleles. Therefore, their minor effects on Ty1 mobility may be due to suppression by secondary mutations in the original isolate or possibly to dependence of the hypermobility phenotype on growth conditions that are particular to the qualitative assay. These possibilities were investigated by quantifying the mobility of a Ty1*his3AI* element in strains containing complete deletions of the *RNR1* and *RTT102* ORFs, which were constructed in the systematic deletion project (Winzeler *et al.* 1999). A Ty1*his3AI-URA3* cassette was integrated upstream of the *HIS4* locus in each strain. The relative frequency of Ty1*his3AI* mobility was increased 156-fold when *RNR1* was deleted (Table 4), which was dramatically higher than the twofold increase in His^+ prototroph formation seen in the *rnr1::*mTn3 strain. These data indicate that a null mutation in *RNR1* results in a tremendous increase in the mobility of Ty1 elements. Therefore, the *rnr1::*mTn3 allele may be partially functional, or the strain may harbor a secondary mutation that partially suppresses Ty1 mobility in the original isolate but that segregates independently in tetrad analysis. On the other hand, an *rtt102*Δ strain displayed no increase in Ty1*his3AI* mobility, suggesting that the apparent hypermobility phenotype of the *rtt102::*mTn3 mutant is restricted to certain assay conditions or is not quantitatively significant.

Mutations in 21 *RTT* genes result in a significant increase in Ty1 mobility: Disruption of the 21 *RTT* genes that were confirmed by tetrad analysis resulted in 5- to 111-fold increases in the relative rate of Ty1 mobility (Figure 3). These 21 *RTT* genes include three previously characterized regulators of Ty1 transposition: *SGS1*, *RAD50*, and *RAD57*. One mutation in an essential gene, *NUT2*, was isolated. The mTn3 insertion is 13 bp upstream of the *NUT2* ORF and therefore probably alters the level of *NUT2* expression. *NUT2* encodes a component of the RNA polymerase II holoenzyme and mediator subcomplex. Another gene encoding a nonessential component of the mediator complex, *MED1*, was also isolated as an *RTT* gene (Table 2). In addition, the previously uncharacterized gene *RTT105* (*YER104W*), which is essential in strain BY4742 but may not be essen-

TABLE 2

Twenty-one regulators of Ty1 transposition

Disrupted *RTT* gene	Strain	Parental strain	Insertion site of mTn3[a] (size of ORF)	Tetrads analyzed	Rate of Ty1*his3AI* mobility (±SE)[b]	Frequency of Ty1*his3AI* mobility (±SD)[c]	Relative cDNA level[d]
—	JC2326	—	—	—	$1.2\ (\pm0.5) \times 10^{-7}$[e]	$3.3\ (\pm2.4) \times 10^{-7}$[f]	1
—	JC2749	—	—	—	$8.9\ (\pm3.4) \times 10^{-8}$[e]	$8.9\ (\pm3.0) \times 10^{-7}$[f]	1
EST2	JC2461	JC2326	+1403 (2654)	10	$5.2\ (\pm0.6) \times 10^{-6}$	$6.7\ (\pm6.7) \times 10^{-5}$	9.4
RRM3	JC2486	JC2326	+163 (2171)	6	$3.4\ (\pm0.9) \times 10^{-6}$	ND	2.3
	JC2832	JC2749	+791 (2171)	—	ND	$1.6\ (\pm0.2) \times 10^{-5}$	6.9
NUT2	JC2639	JC2326	−13 (473)	15	$8.8\ (\pm2.4) \times 10^{-6}$	$3.1\ (\pm0.6) \times 10^{-5}$	6.0
RAD57	JC2817	JC2749	+468 (1382)	6	ND	$1.6\ (\pm0.6) \times 10^{-5}$	3.7
	JC2857	JC2749	+1309 (1382)	6	$7.5\ (\pm2.1) \times 10^{-6}$	ND	7.6
RTT108/YPR164W	JC2837	JC2749	+294 (4223)	—	ND	ND	ND
	JC2388	JC2326	+559 (4223)	11	$3.2\ (\pm0.8) \times 10^{-6}$	ND	3.9
	JC2411	JC2326	+1487 (4223)	—	ND	$2.6\ (\pm1.2) \times 10^{-5}$	5.2
	JC2578	JC2326	+2931 (4223)	—	$3.9\ (\pm1.0) \times 10^{-6}$	ND	7.8
RTT101/YJL047C	JC2410	JC2326	−84 (2528)	—	$2.2\ (\pm0.6) \times 10^{-6}$	ND	2.0
	JC2440	JC2326	−82 (2528)	—	ND	ND	3.0
	JC2412	JC2326	+438 (2528)	7	$2.4\ (\pm0.7) \times 10^{-6}$	$9.4\ (\pm5.0) \times 10^{-6}$	3.2
RRD2	JC2838	JC2749	+41 (1076)	17	$4.0\ (\pm1.2) \times 10^{-6}$	$2.4\ (\pm1.4) \times 10^{-5}$	3.9
RAD50	JC2459	JC2326	+46 (3938)	17	ND	$2.5\ (\pm0.6) \times 10^{-6}$	4.9
	JC2595	JC2326	+187 (3938)	—	$1.5\ (\pm0.4) \times 10^{-6}$	ND	4.7
RTT109/YLL002W	JC2822	JC2749	+996 (1310)	—	ND	$7.7\ (\pm2.0) \times 10^{-6}$	10.8
	JC2812	JC2749	+997 (1310)	6	$2.3\ (\pm0.8) \times 10^{-5}$	$1.6\ (\pm1.2) \times 10^{-5}$	5.0
SGS1	JC2810	JC2749	+201 (4343)	—	$1.6\ (\pm0.4) \times 10^{-6}$	$5.4\ (\pm0.5) \times 10^{-5}$[g]	7.9
	JC2826	JC2749	+795 (4343)	—	ND	ND	ND
	JC2855	JC2749	+1577 (4343)	—	ND	ND	ND
	JC2407	JC2326	+2051 (4343)	—	ND	$1.6\ (\pm0.5) \times 10^{-5}$[g]	1.8
RTT110/YOR144C	JC2642	JC2326	+195 (2375)	12	$5.7\ (\pm1.7) \times 10^{-7}$	$5.6\ (\pm1.9) \times 10^{-6}$	4.7
TEL1	JC2625	JC2326	+1623 (8363)	—	$6.6\ (\pm2.0) \times 10^{-7}$	ND	2.7
	JC2821	JC2749	+5908 (8363)	—	ND	ND	ND
	JC2394	JC2326	+8277 (8363)	13	$1.8\ (\pm0.5) \times 10^{-6}$	ND	1.9
SAE2	JC2479	JC2326	+14 (1037)	10	$1.4\ (\pm0.5) \times 10^{-6}$	$1.3\ (\pm0.2) \times 10^{-6}$	2.3
MED1	JC2824	JC2749	+1116 (1700)	7	$1.3\ (\pm0.4) \times 10^{-6}$	$4.8\ (\pm2.1) \times 10^{-5}$	4.2
RTT103/YDR289C	JC2695	JC2326	+787 (1229)	—	ND	ND	3.7
	JC2389	JC2326	+1023 (1229)	12	$1.5\ (\pm0.5) \times 10^{-6}$	ND	1.6
MRE11	JC2854	JC2749	−3 (2078)	—	ND	ND	ND
	JC2849	JC2749	+278 (2078)	—	ND	$8.0\ (\pm1.2) \times 10^{-6}$	11.7
	JC2811	JC2749	+775 (2078)	11	$1.0\ (\pm0.3) \times 10^{-5}$	$1.3\ (\pm0.5) \times 10^{-5}$	0.8
RTT107/YHR154W	JC2828	JC2749	+299 (3212)	10	$9.2\ (\pm2.8) \times 10^{-6}$	ND	2.4
	JC2393	JC2326	+385 (3212)	3	ND	$2.0\ (\pm1.2) \times 10^{-5}$	2.6
RTT105/YER104W	JC2808	JC2749	+212 (626)	8	$8.4\ (\pm2.6) \times 10^{-6}$	$3.7\ (\pm0.7) \times 10^{-6}$	8.1
SCH9	JC2843	JC2749	+92 (2474)	13	$4.4\ (\pm1.2) \times 10^{-7}$	$2.9\ (\pm2.0) \times 10^{-6}$	1.2
KAP122	JC2834	JC2749	+439 (3245)	8	$5.6\ (\pm1.7) \times 10^{-7}$	$1.6\ (\pm0.5) \times 10^{-6}$	1.7
RTT106/YNL206C	JC2823	JC2749	+186 (1367)	11	$5.7\ (\pm2.0) \times 10^{-7}$	$6.2\ (\pm1.0) \times 10^{-6}$	8.4

ND, not determined.

[a] Number of nucleotides from the beginning of the ORF (+1).

[b] Number of His^+ prototrophs per cell per generation ± standard error.

[c] Number of His^+ prototrophs per cell ± standard deviation.

[d] The amount of Ty1 cDNA relative to parental strain JC2326 or JC2749, as determined by quantitative Southern analysis (see MATERIALS AND METHODS).

[e] The transposition rate of wild-type strains JC2326 and JC2749 was evaluated in successive experiments. Presented here is the result obtained in which the variance was smallest.

[f] Average of values obtained in successive experiments.

[g] Incubation of 20° was on YPD agar rather than YPD broth.

tial in all strains, was isolated (SMITH *et al.* 1996; WINZELER *et al.* 1999).

Several genes with characterized roles in telomere maintenance and/or DNA-damage response were isolated as *RTT* genes, including *EST2*, *TEL1*, *MRE11*, *RAD50*, and *SAE2* (Table 3). *EST2* encodes the catalytic subunit of telomerase. *TEL1* encodes a protein kinase that regulates telomere length and functions in a check-

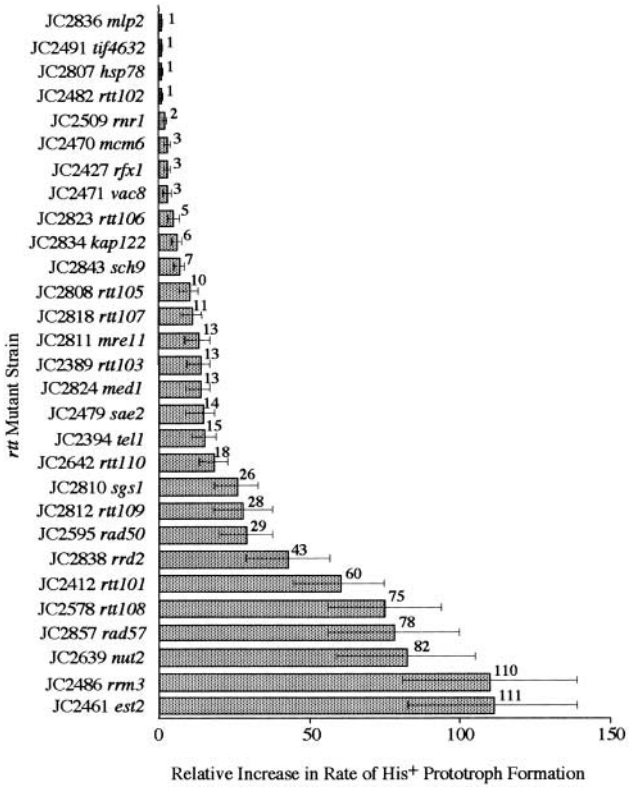

FIGURE 3.—Relative increase in Ty1 cDNA-mediated mobility in 29 *rtt* mutants. The rate of His^+ prototroph formation per cell per generation relative to the isogenic *RTT* strain evaluated in a parallel experiment (*x*-axis) is reported for each *rtt::*mTn3 allele (*y*-axis). The error bars represent ±standard error.

point response to DNA damage. *RAD50* and *MRE11* encode components of the Mre11-Rad50-Xrs2 (MRX) complex, which has multiple roles in genome maintenance, including nonhomologous end-joining, DNA recombinational repair, telomere length regulation, and a DNA-damage checkpoint response. Strains with *rad50*, *mre11*, *xrs2*, or *tel1* mutations exhibit similar telomere shortening phenotypes and are epistatic for telomere length regulation (RITCHIE and PETES 2000). The isolation of *EST2*, *TEL1*, *MRE11*, and *RAD50* as *RTT* genes implies that Ty1 transposition and telomere maintenance may be regulated through a common pathway. Alternatively, the isolation of *SAE2*, which encodes a modulator of MRX complex activity in DNA repair, raises the possibility that *TEL1*, *MRE11*, *RAD50*, and *SAE2* all regulate Ty1 transposition through the Tel1-Mre11 checkpoint pathway (USUI *et al.* 2001).

Another class of *RTT* genes encodes proteins that suppress DNA recombination between repeated sequences, including *SGS1*, *RRM3*, and *RTT110* (Table 3). Sgs1 suppresses rDNA recombination, Y′ subtelomeric repeat recombination, and extrachromosomal Ty1 cDNA recombination. Rrm3 is a superfamily I DNA helicase believed to be the replicative helicase for rDNA. Rrm3 inhibits recombination between rDNA repeats and promotes telomere replication. *RTT110* has been identified by another group as *EFD1*, encoding a protein that inhibits direct repeat recombination between LTRs of a Ty1 element and repeats created by plasmid integration (S. BEN-AROYA, B. LIEFSHITZ and M. KUPIEC, personal communication).

Another *RTT* gene, *RRD2*, together with its homolog *RRD1*, encodes a putative phosphotyrosyl phosphatase activator (Table 3). Rrd2 interacts genetically with the high osmolarity pathway kinase Hog1, which was previously shown to inhibit Ty1 transposition (CONTE and CURCIO 2000). *RTT101* is ORF *YJL047C*. It encodes one of four cullin homologs in yeast, which are components of the Skp1-Cullin-F-box complex (SCF) family of E3 ubiquitin ligases. It has recently been shown that Rtt101 is modified by covalent attachment to the ubiquitin-like protein Rub1, but Rub1 is not involved in regulation of Ty1 transposition (J. M. LAPLAZA, M. BOSTICK, D. T. SCHOLES, M. J. CURCIO and J. CALLIS, unpublished results). *KAP122* encodes a nuclear transport factor and *SCH9* encodes a kinase in a stress response and nutrient-sensing signaling pathway. Disruption of either *KAP122* or *SCH9* has a relatively modest effect on Ty1 mobility (Figure 3). Six additional genes with uncharacterized functions were demonstrated to regulate Ty1 transposition. *RTT107* is ORF *YHR154C*, which belongs to a family of BRCT-domain proteins with characterized or putative roles in cell cycle checkpoint pathways responsive to DNA damage. *RTT103* (*YDR289C*), *RTT106* (*YNL206C*), *RTT108* (*YPR164W*), and *RTT109* (*YLL002W*) have no known homologs.

We determined whether increased Ty1 cDNA-mediated mobility was the phenotype of null mutations in 11 of the 21 *RTT* genes, using strains that contain complete deletions of the *RTT* ORFs. The Ty1*his3AI[Δ1]-URA3* cassette was integrated into each *rtt*Δ strain and the isogenic wild-type strain, BY4742. In 10 *rtt*Δ strains tested, including *est2*Δ, *kap122*Δ, *med1*Δ, *mre11*Δ, *rrm3*Δ, *rtt107*Δ, *rtt109*Δ, *rtt110*Δ, *sae2*Δ, and *tel1*Δ, there was a 4- to 34-fold increase in Ty1*his3AI* mobility relative to the wild-type strain. Most of the *rtt*Δ mutations result in equivalent or less severe hypermobility phenotypes than the corresponding *rtt::*mTn3 allele. This may be because the Ty1*his3AI[Δ]* element integrated into BY4742 has a higher rate of mobility than the Ty1*his3AI*-270 element in strains JC2749 and JC2326. In contrast to other *rtt*Δ strains, Ty1*his3AI* mobility was not significantly increased in an *rrd2*Δ strain. *RRD2* is one of two functionally redundant homologs in yeast, and an *rrd2*Δ mutation results in only mild phenotypes except when combined with *rrd1*Δ (REMPOLA *et al.* 2000). This result may indicate that the function of *RRD1* is compromised in the JC2749 strain, but not in the BY4742 strain in which the phenotype of deletion alleles was tested, resulting in a 43-fold increase in Ty1 mobility in the *rrd2::*mTn3 mutant.

***RTT* genes regulate post-transcriptional steps in Ty1**

TABLE 3

Identified *RTT* genes and their functions

Gene name	ORF	Gene product function	Reference
		Marginal regulators	
HSP78	*YDR258C*	ATP-dependent protease, mitochondrial protein chaperone	Leonhardt *et al.* (1993); Schmitt *et al.* (1995)
MCM6	*YGL201C*	Component of hexameric helicase complex involved in DNA replication	Reviewed in Tye (1999)
MLP2	*YIL149C*	Nuclear envelope protein that interacts with Yku70	Strambio-de-Castillia *et al.* (1994); Galy *et al.* (2000)
RFX1	*YLR176C*	Repressor of DNA damage-inducible genes	Huang *et al.* (1998)
RNR1	*YER070W*	Large subunit of ribonucleotide reductase	Elledge and Davis (1990)
RTT102	*YGR275W*	Unknown, not similar to any protein of known function	Fiori *et al.* (2000)
TIF4632	*YGL049C*	One of two eIF4G homologues in yeast	Goyer *et al.* (1993)
VAC8	*YEL013W*	Vacuolar protein involved in protein targeting	Wang *et al.* (1996)
		Regulators	
EST2	*YLR318W*	Catalytic subunit of telomerase	Lendvay *et al.* (1996)
KAP122	*YGL016W*	Member of karyopherin-beta family, component of nuclear pore complex	Titov and Blobel (1999)
MED1	*YPR070W*	Component of Pol II transcription mediator complex	Balciunas *et al.* (1999)
MRE11	*YMR224C*	Component of the Mre11-Rad50-Xrs2 (MRX) complex, with role in nonhomologous end-joining, DNA repair, telomere length regulation, TM checkpoint pathway	Reviewed in Haber (1998); Usui *et al.* (2001)
NUT2	*YPR168W*	Component of Pol II transcription mediator complex	Gustafsson *et al.* (1998); Tabtiang and Herskowitz (1998)
RAD50	*YNL250W*	See *MRE11*; previously identified as regulator of Ty1 transposition	Rattray *et al.* (2000)
RAD57	*YDR004W*	RecA homologue stimulating strand-exchange activity of Rad51 during homologous recombination; previously identified as regulator of Ty1 transposition	Sung (1997); Rattray *et al.* (2000)
RRD2	*YPL152W*	Encodes putative phosphotyrosyl phosphatase activator	Rempola *et al.* (2000)
RRM3	*YHR031C*	Superfamily I DNA helicase required for replication fork progression in rDNA	Ivessa *et al.* (2000)
RTT101	*YJL047C*	Cullin, putative component of the Skp1-Cullin-F-box complex (SCF) family of E3 ubiquitin ligases	Ohta *et al.* (1999)
RTT103	*YDR289C*	Unknown, not similar to any protein of known function	
RTT105	*YER104W*	Unknown, not similar to any protein of known function	
RTT106	*YNL206W*	Unknown, similarity to DNA structure-specific recognition protein (SSRPs)	Jonniaux *et al.* (1994)
RTT107	*YHR154W*	Unknown, BRCT-domain protein family	Bork *et al.* (1997)
RTT108	*YPR164W*	Unknown, mutant sensitive to diepoxybutane and mitomycin C	Saccharomyces Genome Database
RTT109	*YLL002W*	Unknown, mutant sensitive to diepoxybutane and mytomycin C	Saccharomyces Genome Database
RTT110	*YOR144C*	Inhibits direct repeat recombination	S. Ben-Aroya, B. Liefshitz and M. Kupiec, personal communication
SAE2	*YGL175C*	Regulator of the activity of the MRX complex	Rattray *et al.* (2001); Usui *et al.* (2001)
SCH9	*YHR205W*	Kinase in stress response and nutrient-sensing signaling pathway	Toda *et al.* (1988); Fabrizio *et al.* (2001)
SGS1	*YMR190C*	RecQ-family helicase that suppresses recombination; previously identified as regulator of Ty1 transposition	Gangloff *et al.* (1994); Watt *et al.* (1996); Bryk *et al.* (2001)
TEL1	*YBL088C*	Protein kinase required for telomere length regulation and TM checkpoint pathway	Greenwell *et al.* (1995); Morrow *et al.* (1995); Usui *et al.* (2001)

TABLE 4

Frequency of Ty1*his3AI[ΔI]* mobility in *rtt*Δ strains

Experiment	Relevant genotype	Frequency of Ty1*his3AI[Δ1]* mobility[a] ±SD	Relative Ty1 mobility frequency[b]
I	WT	$5.2 \pm 0.9 \times 10^{-7}$	1
	*rtt110*Δ	$8.8 \pm 0.5 \times 10^{-6}$	17
	*rtt107*Δ	$6.0 \pm 1.4 \times 10^{-6}$	12
	*rrm3*Δ	$1.4 \pm 0.3 \times 10^{-5}$	27
	*mre11*Δ	$4.6 \pm 0.8 \times 10^{-6}$	9
	*tel1*Δ	$1.9 \pm 0.5 \times 10^{-6}$	4
	*rnr1*Δ	$8.6 \pm 2.1 \times 10^{-5}$	156
II	WT	$4.9 \pm 1.5 \times 10^{-7}$	1
	*rtt109*Δ	$1.6 \pm 0.2 \times 10^{-5}$	34
	*kap122*Δ	$3.7 \pm 1.1 \times 10^{-6}$	8
III	WT	$3.0 \pm 1.8 \times 10^{-7}$	1
	*rtt102*Δ	$4.5 \pm 1.9 \times 10^{-7}$	1.5
	*est2*Δ	$4.4 \pm 2.0 \times 10^{-6}$[c]	11
	*rrd2*Δ	$4.1 \pm 2.7 \times 10^{-7}$	1.4
	*med1*Δ	$1.6 \pm 0.8 \times 10^{-6}$[c]	4
	*sae2*Δ	$1.6 \pm 0.5 \times 10^{-6}$	5

[a] Number of His^+ Ura^+ prototrophs divided by the total number of Ura^+ cells analyzed. Value presented is the average frequency ± standard deviation calculated from between four and six independent cultures grown at 20°.

[b] Frequency of Ty1*his3AI[Δ1]* mobility relative to that of the wild-type strain BY4742.

[c] The frequency of Ty1*his3AI[Δ1]* mobility was evaluated after 7 days growth of colonies. This was compared with the strain BY4742 frequency evaluated after 7 days growth, which was $3.9 \pm 1.3 \times 10^{-7}$.

retrotransposition: To determine whether *RTT* gene products affect the expression of Ty1 elements or the stability of Ty1 mRNA, the relative levels of Ty1 RNA and Ty1*his3AI* RNA in *rtt* mutants were determined. Strains harboring mTn3 disruptions of the 21 *RTT* genes that repress Ty1 cDNA-mediated mobility more than fivefold were analyzed by Northern blotting. In the case of multiple insertion alleles of the same *RTT* gene, RNA was quantitated from the same strain that was used to determine the relative Ty1 mobility rate (Figure 3). The level of Ty1*his3AI* RNA in *rtt* mutants was between 0.4- and 2.8-fold that of the isogenic wild-type strain (Figure 4, top). Similarly, Ty1 RNA in *rtt* mutants was 0.4- to 2.0-fold the level in the corresponding wild-type strain (Figure 4, middle). As a control, Ty1 and Ty1*his3AI* transcripts were shown to be markedly reduced in a *tec1* strain, which is defective for expression of Ty1 elements (Laloux *et al.* 1990). Hence, the data suggest that increased Ty1 mobility is not due to elevated levels of Ty1 or Ty1*his3AI* RNA in the *rtt::*mTn3 strains. One exception may be the *rtt103* mutant, which displayed a 2.8-fold increase in the ratio of Ty1*his3AI/PYK1* RNA and a 1.8-fold increase in the ratio of Ty1/*PYK1* RNA (Figure 4). These small increases in RNA are associated with a 13-fold increase in Ty1*his3AI* mobility (Figure 3). Hence, elevated Ty1 RNA levels may contribute to elevated Ty1 mobility in *rtt103* mutants. In summary, the results of Northern analysis suggest that most or all of the 21 *RTT* gene products exert their primary effect on post-transcriptional steps in Ty1 retrotransposition.

Ty1 cDNA levels are elevated in most *rtt* mutants: To determine whether the elevated rates of Ty1 mobility are correlated with increases in a physical intermediate in transposition in *rtt::*mTn3 mutants, we quantified unintegrated linear Ty1 cDNA in strains harboring mTn3 insertion alleles of all 29 *RTT* genes isolated in the screen using a quantitative Southern blot assay (Bryk *et al.* 2001). Total cellular DNA digested with *Pvu*II was hybridized to a radiolabeled *TYB1* probe (Figure 5A). The probe detects a 2.0-kb fragment of Ty1 cDNA from a conserved *Pvu*II site in Ty1 to the 3′ end of the linear extrachromosomal cDNA. In addition, the probe detects numerous *Pvu*II fragments >2.0 kb, each of which represents a unique junction between the 3′ end of a genomic Ty1 element and flanking DNA. Ty1 cDNA levels were determined by quantifying the intensity of the 2.0-kb Ty1 cDNA band (Figure 5B, band C) relative to the intensities of two genomic Ty1 junction bands (Figure 5B, bands G1 and G2) in two or three independent DNA samples from each *rtt* strain. Of the 8 *rtt::*mTn3 strains that displayed a ≤3-fold increase in Ty1 mobility (Table 1), 7 had Ty1 cDNA levels <2.0-fold greater that of the isogenic wild-type strain. The 8th strain, which harbors *rnr1::*mTn3, had a 2.3-fold increase in Ty1 cDNA. In addition, we measured relative cDNA levels in 34 *rtt::*mTn3 strains that displayed a ≥5-fold increase in Ty1 mobility (Table 2). Twenty-eight mTn3 disruptions in 19 *RTT* genes caused levels of Ty1 cDNA to be increased 2.0- to 11.7-fold. The results indicate that the increased Ty1 mobility in most *rtt* mutants is correlated with elevated Ty1 cDNA levels, suggesting that most Rtt proteins suppress cDNA levels. This may occur by direct inhibition of Ty1 cDNA synthesis or stability or by inhibition of an earlier step in retrotransposition that indirectly results in low cDNA levels.

Six of the 34 *rtt::*mTn3 strains analyzed displayed an increase in Ty1 cDNA that was <2-fold (Table 2). These included the *sch9-92::*mTn3 and *kap122-439::*mTn3 mutants, in which the rate of Ty1 mobility is elevated only 7-fold and 6-fold, respectively (Figure 3). In addition, strains harboring mTn3 insertions in *SGS1*, *TEL1*, *RTT103*, and *MRE11* displayed a <2.0-fold increase in Ty1 cDNA, but strains harboring different mTn3 insertions closer to the 5′ end of each of these ORFs had Ty1 cDNA levels that were elevated 2.7- to 11.7-fold (Table 2). The location of mTn3 in these ORFs affected cDNA levels but did not significantly change the hypermobility phenotype (Table 2). Hence, modulation of Ty1 cDNA levels may not be the primary mechanism by which these proteins inhibit Ty1 mobility.

Mutations in *RTT* genes have varied effects on Ty1

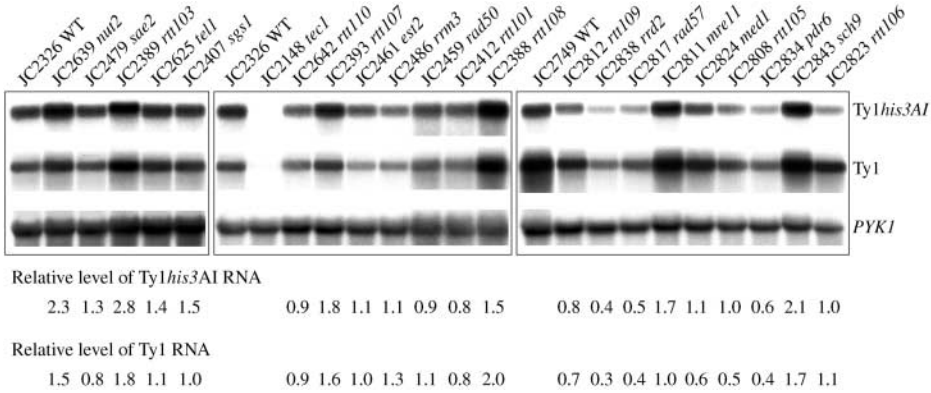

FIGURE 4.—Ty1*his3AI* RNA and total Ty1 RNA levels in 21 *rtt* mutants. Northern analyses of three blots of total RNA from *rtt* mutant and control strains hybridized sequentially to ^{32}P-riboprobes for Ty1*his3AI*, Ty1, and *PYK1* RNA are shown. The relative levels of Ty1*his3AI* RNA and Ty1 RNA are the ratios of Ty1*his3AI*/*PYK1* RNA and Ty1/*PYK1* RNA, respectively, compared to the corresponding ratios of the isogenic wild-type strain analyzed on the same membrane.

integration: Given that most *rtt* mutants have elevated levels of Ty1 cDNA, we tested the hypothesis that *de novo* integration events are also stimulated. We employed a PCR-based assay to detect *de novo* integration of Ty1 elements upstream of 16 glycyl-tRNA genes in *rtt* mutants. The targets were chosen because at least 1 glycyl-tRNA gene (the *SUF16* locus on chromosome III) is a hotspot for Ty1 transposition (JI *et al.* 1993). PCR was performed using one primer containing *TYB1* sequence and one primer containing glycyl-tRNA sequence. Ty1 transposition events ~100–800 bp upstream of and in the same transcriptional orientation as glycyl-tRNA genes yielded PCR products ranging from ~0.55 to 1.2 kb (Figure 6). The observed periodicity of Ty1 integration events may be attributable to phased nucleosomes or another chromatin feature specific to the vicinity of tRNA genes (VOYTAS and BOEKE 2002). An increase in the intensity and number of PCR products indicated that *de novo* integration events were elevated. Control DNA samples from a *tec1* mutant, which has a hypotransposition phenotype, yielded low levels of PCR products, whereas those from an *ssl2-rtt* mutant, which has a strong hypertransposition phenotype (LEE *et al.* 1998), yielded high levels of PCR products.

Genomic DNA from four independent cultures of seven *rtt::*mTn3 mutants were analyzed. The *nut2::*mTn3 and *rtt101::*mTn3 mutants dramatically increased the level of Ty1 integration relative to the wild-type strain (Figure 6). These mutants have 82- and 60-fold higher levels of Ty1*his3AI* mobility and 6- and 3.2-fold higher levels of Ty1 cDNA, respectively, compared to the wild-type strain. Therefore, it is likely that these mutations affect the accumulation of an intermediate in Ty1 transposition, such as Ty1 cDNA or VLPs, which directly results in increased transposition. In contrast, no increase in integration upstream of glycyl-tRNA genes was detected in *tel1::*mTn3 or *rrm3::*mTn3 mutants. The PCR assay may be too insensitive to detect the 15-fold increase in cDNA mobility in the *tel1::*mTn3 mutant. However, the *rrm3::*mTn3 mutant showed a 110-fold increase in Ty1 cDNA-mediated mobility (Figure 3). These results suggest that mutations in *RRM3* cause Ty1 cDNA to be processed differently from that in wild-type cells. For example, high levels of Ty1 cDNA recombination or cDNA integration at novel target sites could explain the paradoxical increase in Ty1 mobility in the absence of integration upstream of glycyl-tRNA genes.

A modest increase in the level of integration, with variability between samples, was seen in *est2::*mTn3, *rtt108::*mTn3, and *rad50::*mTn3 mutants, which had 111-fold, 75-fold, and 29-fold increases in Ty1 mobility, respectively. The data suggest that an increase in cDNA

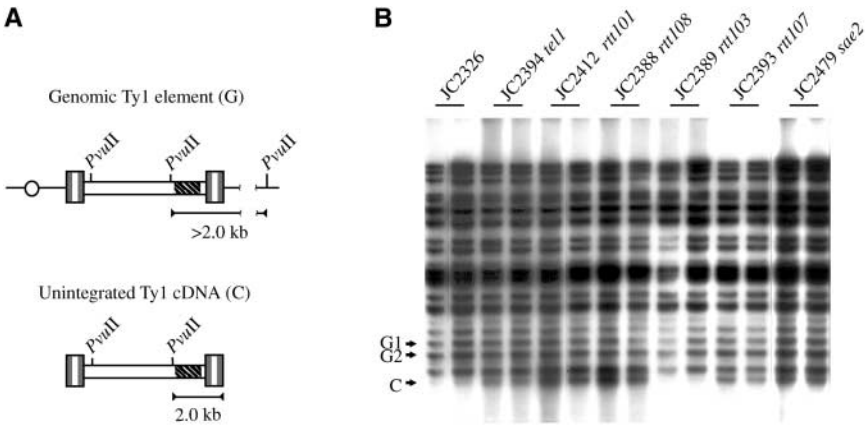

FIGURE 5.—Relative levels of unintegrated Ty1 cDNA in *rtt* mutants. (A) Diagrams of unintegrated Ty1 cDNA and a genomic Ty1 element, indicating the location of the *TYB1* hybridization probe (hatched box) and relevant *Pvu*II cleavage sites. The *TYB1* probe detects a 2.0-kb *Pvu*II fragment of unintegrated Ty1 cDNA and variably sized >2.0-kb fragments containing the junction of Ty1 elements with chromosomal DNA at different locations. (B) Southern blot analysis of *Pvu*II-digested total cellular DNA from cells grown at 20°. Each DNA sample was extracted from a culture inoculated with an independent colony. The ratio of ^{32}P activity in the 2.0-kb cDNA band (band C) relative to the activity in two genomic Ty1 bands (bands G1 and G2) was calculated for each DNA sample, and the average of two to three DNA samples is reported in Table 1.

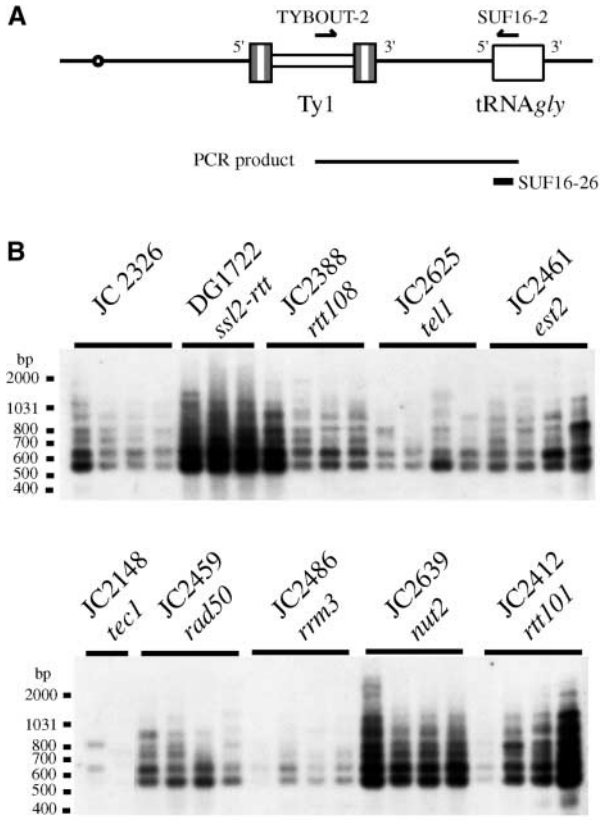

FIGURE 6.—Detection of unselected Ty1 integration events upstream of glycyl-tRNA genes, a preferred integration target. (A) Diagram of a Ty1 transposition event upstream of 1 of 16 glycyl-tRNA genes in the same transcriptional orientation. Primer SUF16-2 anneals to the glycyl-tRNA gene, and primer TYBOUT-2 anneals to the 3′ end of *TYB1* (indicated by arrows). A PCR product of a *de novo* Ty1 insertion upstream of a glycyl-tRNA gene in the orientation shown is detected using a radiolabeled SUF16-26 probe. (B) Southern blot of PCR amplification of genomic DNA samples from independent cultures of each strain. A wild-type strain, *ssl2-rtt* mutant (positive control), *tec1* mutant (negative control), and seven *rtt* mutants are shown.

integration at preferred target sites is probably a significant component of elevated cDNA mobility in these mutants. However, other pathways of cDNA mobility, such as integration at alternative target sites or recombination, may also be stimulated. In summary, these data suggest that some Rtt factors repress Ty1 mobility by reducing the amount of a physical intermediate in transposition, whereas others may alter the fate of Ty1 cDNA.

Inhibition of Ty1 cDNA-mediated mobility by multiple regulators of telomere replication: Since Est2, a subunit of telomerase, and the telomere length regulators, Tel1, Mre11, and Rad50, were found to inhibit the mobility of Ty1, we postulated that transpositional dormancy is linked to telomere maintenance. To explore this possibility, the Ty1*his3AI[Δ1]-URA3* cassette was introduced into derivatives of strain BY4742 harboring deletions of five additional ORFs required for telomere maintenance, and Ty1*his3AI* mobility was analyzed.

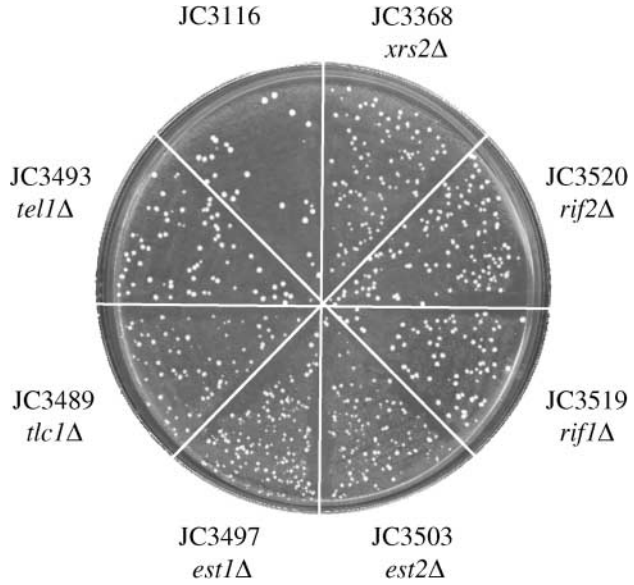

FIGURE 7.—Genes encoding regulators of telomere length are required to repress Ty1 cDNA-mediated mobility. Each strain harbors the integrated Ty1*his3AI[Δ1]*-URA3 cassette and was grown as a patch on one-eighth of an SC-Ura plate at 30°, replicated to YPD and grown for 3 days at 20°, and then replicated to SC-Ura-His and grown for 3 days at 30°. The experiment was repeated four times, and one representative plate is shown here. His$^+$ papillae are formed from individual cells that sustain a Ty1*HIS3* cDNA mobility event.

TLC1, which encodes the RNA subunit of telomerase (SINGER and GOTTSCHLING 1994), and *EST1*, which encodes another component of the telomerase holoenzyme (LUNDBLAD and SZOSTAK 1989), are in the same epistasis group as *EST2* for telomere maintenance (LENDVAY *et al.* 1996). Deletion of *TLC1* or *EST1* resulted in a significant increase in His$^+$ prototroph formation, similar to that observed in an *est2*Δ strain (Figure 7). Deletion of *XRS2*, which encodes a third component of the MRX complex, also increased His$^+$ prototroph formation. Interestingly, *rif1*Δ and *rif2*Δ mutant strains exhibited increased His$^+$ prototroph formation as well. *RIF1* and *RIF2* encode Rap1-interacting proteins. In contrast to the other strains tested, strains lacking Rif1 or Rif2 have elongated telomeres (MARCAND *et al.* 1997; WOTTON and SHORE 1997). Taken together, these findings support the hypothesis that signaling pathways that sense and respond to telomere length also regulate Ty1 mobility.

DISCUSSION

Numerous yeast genes encode regulators of Ty1 transposition: Here we describe the identification and preliminary characterization of 21 *RTT* genes. The use of transposon-mediated mutagenesis allowed *RTT* genes to be identified on the basis of their hypermobility phenotype for the first time. We assume that these 21 genes represent only a fraction of the *RTT* genes in yeast,

because the 10,000 mTn3-*lacZ/LEU2* transformants that we analyzed were only about one-third the number required to represent the entire genome (Ross-MacDonald *et al.* 1998). Furthermore, only 112 of the 274 mutants that we isolated were characterized. Accordingly, not all of the previously known regulators of Ty1 transposition were identified in our screen. Transposition-mediated mutational analysis is biased against the detection of essential genes, and therefore we did not expect to isolate mutations in genes encoding essential regulators of Ty1 transposition, including *SSL2*, *RAD3*, or *CDC9*. However, mutations in *FUS3*, *HOG1*, *RAD51*, *RAD52*, and *RAD54* were expected but not isolated. Hence, our study, together with previous studies (Conte *et al.* 1998; Lee *et al.* 1998, 2000; Conte and Curcio 2000; Rattray *et al.* 2000), demonstrates that a very large number of host factors inhibit Ty1 transposition directly or indirectly. This finding reveals an interdependent and evolutionarily refined relationship between Ty1 and its host cell.

Because of the large number of genes that regulate transposition, secondary mutations that enhance the hypermobility phenotype of a mutation may arise at a high frequency. Hence, as our analysis has revealed, it is necessary to confirm that the hypermobility phenotype of a mutant is due to a single gene mutation. Approximately one-third of the *rtt::*mTn3 alleles that we isolated failed to show 2:2 segregation of the hypermobility phenotype in tetrad analysis. Instead, hypermobility was rare or completely absent among the progeny of these *rtt::*mTn3 mutants. The simplest interpretation is that preexisting mutations or mutations introduced during transformation contributed to the hypermobility associated with these *rtt::*mTn3 alleles in the original isolate.

The presence of secondary mutations that dampen the hypermobility phenotype of *rtt* mutations may also complicate the analysis of *RTT* genes. Eight different *rtt* mutants showed 2:2 segregation of a qualitative hypermobility phenotype in tetrad analysis, but the original isolate did not show elevated His^+ prototroph formation in a quantitative assay (Table 1). These seemingly contradictory results could be attributable to partially functional *rtt::mTn3* alleles or to the presence of a partially suppressing secondary mutation in the original *rtt::*mTn3 mutant. One of these explanations is likely to apply to the *rnr1::mTn3* mutant phenotype, since an *rnr1*Δ mutant showed a dramatically higher level of Ty1 mobility in a quantitative assay (Table 4) than the *rnr1::*mTn3 mutant (Figure 3). Other *rtt::*mTn3 mutations may cause an increase in Ty1 mobility under particular environmental conditions that differ when cells are grown on agar as opposed to a liquid medium. These may include the availability of nutrients and proximity of cells to each other.

***RTT* genes act primarily at post-transcriptional steps in Ty1 mobility:** Of the 21 *RTT* genes whose products inhibit Ty1 mobility fivefold or more (Table 2), none caused an increase in Ty1 RNA levels of greater than twofold when disrupted. This finding indicates that Rtt factors exert their effect on Ty1 mobility primarily at steps following transcription or mRNA degradation. Our analysis does not rule out the possibility of transcriptional regulation of Ty1 elements, but does highlight the prevalence of post-transcriptional mechanisms in maintaining transpositional dormancy. The isolation of post-transcriptional regulators was anticipated, given the unusually high level of Ty1 mRNA and the paradoxically low levels of Ty1 VLPs, cDNA, and transposition. We propose the following model to explain why regulation of transposition at post-transcriptional levels may be predominant over transcriptional regulation. If the host represses Ty1 transposition by inhibiting Ty1 element expression or RNA stability, a selective advantage is conferred upon an individual Ty1 element that sustains genetic alterations that allow it to evade that repression. This element would transpose preferentially, because its RNA would represent a larger fraction of the total Ty1 RNA pool. Consequently, the proportion of elements that could evade transcriptional repression by the host would increase over time. In contrast, if the host regulates Ty1 mobility at a post-transcriptional level, for instance, by destabilizing a Ty1 protein, less advantage is conferred upon an individual Ty1 element that can evade this repression. This is because Ty1 proteins act efficiently *in trans* (Xu and Boeke 1990; Curcio and Garfinkel 1992; Curcio and Garfinkel 1994), so the stabilized protein would activate all Ty1 elements. Therefore, the altered Ty1 would not transpose preferentially relative to other elements. Consequently, there is less selective pressure on individual Ty1 elements to evade post-transcriptional repression than to evade transcriptional repression. It follows that regulation of Ty1 transposition at the post-transcriptional level is more likely to be successfully sustained by the host.

The increased cDNA levels in the absence of increased Ty1 RNA levels observed in most *rtt* mutants demonstrate that many *RTT* gene products inhibit Ty1 transposition at a post-transcriptional and preintegrational stage of the Ty1 retrotransposition cycle. In general, *rtt* mutants with higher levels of Ty1 cDNA exhibit higher levels of Ty1 mobility. For example, only a 3.3-fold average increase in cDNA was observed in 10 *rtt* mutants in which the Ty1 mobility rate was elevated 5- to 15-fold, whereas 11 *rtt* mutants with an 18- to 111-fold increase in Ty1 mobility rate had a 5.7-fold increase in cDNA. However, there are specific examples of *rtt* mutants in which this correlation is violated. For example, the *rtt106::*mTn3 mutant strain displayed an 8.4-fold increase in Ty1 cDNA but only a 5-fold increase in mobility. Perhaps inactivation of Rtt106, which has similarity to DNA structure-specific recognition proteins (SSRPs; Table 3), results in the accumulation of Ty1 cDNA that cannot be recognized by the Ty1 IN protein.

On the other hand, some *rtt* mutants display a large increase in Ty1 mobility but do not have dramatically increased cDNA levels. For example, an *rrm3* mutant exhibited a 110-fold increase in Ty1 mobility yet had only a 2.3-fold increase in Ty1 cDNA.

On the basis of Ty1 cDNA quantitation and analysis of *de novo* integration upstream of tRNA genes, the *RTT* genes isolated to date fall into at least two classes. The first class consists of genes whose products directly or indirectly reduce the levels of physical intermediates required in the transposition process. These intermediates may include Ty1 proteins, Ty1 cDNA, or host factors required for transposition. The previously characterized *RTT* genes *SSL2*, *RAD3*, and *FUS3* fall into this class, and the newly identified genes *NUT2* and *RTT101* are both likely members. In *nut2* and *rtt101* mutants, significant increases in Ty1 cDNA levels were detected, and frequent integration upstream of glycyl-tRNAs was observed. These findings suggest that transposition occurs more efficiently in *nut2* and *rtt101* mutants. Given that Rtt101 encodes a component of an E3-ubiquitin ligase, it is possible that Ty1 proteins are modified by an Rtt101-containing complex, leading to their degradation or an alteration in their activity. Nut2, together with Med1, is a component of the Pol II transcription mediator complex. Hence, mutations in *nut2::*mTn3 and *med1::*mTn3 may cause a defect in the expression of a host factor required for Ty1 transposition. Alternatively, Nut2 and Med1 may have secondary roles outside of transcription regulation. Notably, the mediator complex strongly stimulates TFIIH to phosphorylate the C-terminal domain of the largest subunit of RNA Polymerase II (Kim *et al.* 1994; Myers *et al.* 1998). Ssl2 and Rad3 are components of TFIIH and potent activators of Ty1 cDNA degradation. Perhaps the mediator complex also activates TFIIH, or a subcomplex containing Ssl2 and Rad3, to promote cDNA degradation, thereby repressing Ty1 transposition.

A second class of *RTT* genes includes those that inhibit alternative pathways of cDNA mobility, including homologous recombination or integration at novel target sites. This class is typified by mutations that cause an increase in cDNA mobility but do not show a corresponding increase in integration of Ty1 upstream of glycyl-tRNA genes. This class includes *SGS1* and probably *RRM3* as well. Notably, *rrm3* and *sgs1* mutants have similar hypermobility phenotypes, including significantly elevated levels of Ty1 mobility, allele-dependent variations in Ty1 cDNA levels (Table 2), and no effect on integration upstream of glycyl-tRNA genes (Figure 6; Bryk *et al.* 2001). Moreover, both genes encode helicases associated with DNA replication and both repress recombination between rDNA repeats and other directly repeated sequences. Hence, Rrm3 may repress transposition by the same mechanism as Sgs1.

Mutations in *EST2*, *RTT108*, and *RAD50* result in an intermediate phenotype in the assay for integration upstream of tRNAs, despite the fact that these mutations cause 111-fold, 75-fold, and 29-fold increases in Ty1 mobility, respectively. Mutations in all three genes also result in a significant increase in Ty1 cDNA. At present, we cannot conclude whether the primary cause of the hypermobility in these mutants is the increase in Ty1 transposition intermediates, or an altered cDNA fate, or both. In the case of the *est2::*mTn3 mutant, it is possible that the cells grown to assay Ty1 mobility had a different telomere structure from those grown to quantify cDNA integration, and different telomere structures may have resulted in different levels of Ty1 transposition in each population. When *EST2* is disrupted, cells show progressive shortening of telomeres and progressive loss of viability (Lendvay *et al.* 1996). Cells with two distinct telomeric structures (type I and type II) arise rarely from the senescing populations (Teng and Zakian 1999). We are presently investigating whether and how Ty1 mobility is affected by senescence and by the different growth characteristics of type I and type II *est2*Δ survivors.

Potential links between Ty1 transposition and the response to DNA damage: We have shown that Est2, the catalytic subunit of telomerase, inhibits Ty1 mobility at a post-transcriptional level. Est2 may act directly or indirectly to repress Ty1 transposition. The latter possibility seems more likely because other genes involved in telomere maintenance were identified as putative or proven *RTT* genes. For example, mutations in other genes required for telomerase function, including *TLC1* and *EST1*, result in Ty1 hypermobility (Figure 7). Furthermore, mutations in *TEL1*, which cause telomere shortening, and mutations in *RIF1* and *RIF2*, which cause telomere lengthening, result in Ty1 hypermobility (Figures 3 and 7). Taken together, these findings suggest that alterations in the normal telomere structure may act as signals that result in the activation of Ty1 transposition.

In addition to the *RTT* genes discussed above, several other genes that play roles in genome maintenance were identified, including *MRE11*, *RAD50*, *RAD57*, *SAE2*, *RTT110*, *SGS1*, *RRM3* (Figure 3), *XRS2* (Figure 7), and *RNR1* (Table 4). Some of these *RTT* gene products may bind to Ty1 cDNA directly and influence its fate. For example, the MRX complex is known to bind and process DNA double-strand breaks. Perhaps the MRX complex binds to the free ends of Ty1 cDNA and prevents integration or gene conversion of genomic Ty1 elements by promoting cDNA degradation or altering cDNA structure. A second possibility is that some of these *RTT* gene products are involved in sensing unprotected Ty1 cDNA ends in the nucleus and generating a response. For example, recognition of Ty1 cDNA by the MRX complex could result in activation of the Tel1-Mre11 checkpoint pathway, and this pathway may activate an inhibitor of Ty1 mobility. If so, it is likely that Tel1 and Sae2, a modulator of the activity of the Tel1-

Mre11 checkpoint pathway, inhibit transposition by the same mechanism.

Another way in which mutations of some *RTT* genes may affect transposition is by creating DNA lesions that activate a DNA-damage response pathway, which in turn activates Ty1 mobility. Ty1 transcript and transposition levels are increased in response to some types of DNA damage (Rolfe and Banks 1986; Bradshaw and McEntee 1989; Morawetz and Hagen 1990; Staleva Staleva and Venkov 2001). Perhaps DNA-damage response pathways stimulate Ty1 transposition at post-transcriptional levels as well. One *RTT* gene that may act in this way is *RNR1*. The transcriptional profile of an *rnr1*Δ mutant is similar to that of cells exposed to hydroxyurea, suggesting that deletion of *RNR1* mimics a stress response (Hughes *et al.* 2000). Induction of this stress response pathway by deletion of *RNR1* may derepress Ty1 transposition.

The regulation of Ty1 transposition by a large number of conserved proteins involved in genome maintenance and other cellular pathways suggests that yeast have adapted to the presence of Ty1 elements in the genome in such a way that their mutagenic potential is harnessed. Ty1 retrotransposition has several potentially deleterious effects, including gene disruption and gross chromosomal deletions and rearrangements resulting from recombination between elements at ectopic sites. Hence, Ty1 elements can be viewed as having a largely negative role in the genome. On the other hand, their ability to cause regulatory mutations that allow rapid adaptation to new environments suggests that Ty1 retrotransposons may also have a positive evolutionary role (reviewed in Boeke and Sandmeyer 1991; Wilke and Adams 1992). McClintock (1984) proposed that one role of transposons may be to promote genome reorganization at times when the cell is exposed to genomic shock or other types of stress, so that adaptively favorable mutations might arise. Our demonstration that Ty1 mobility is regulated by numerous conserved gene products required for stability of the genome suggests the hypothesis that Ty1 elements can be activated by certain types of injury to the genome through DNA-damage signaling pathways.

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