Host co-factors of the retrovirus-like transposon Ty1

Curcio et al.
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Abstract

Background: Long-terminal repeat (LTR) retrotransposons have complex modes of mobility involving reverse transcription of their RNA genomes in cytoplasmic virus-like particles (VLPs) and integration of the cDNA copies into the host genome. The limited coding capacity of retrotransposons necessitates an extensive reliance on host co-factors; however, it has been challenging to identify co-factors that are required for endogenous retrotransposon mobility because retrotransposition is such a rare event.

Results: To circumvent the low frequency of Ty1 LTR-retrotransposon mobility in Saccharomyces cerevisiae, we used iterative synthetic genetic array (SGA) analysis to isolate host mutations that reduce retrotransposition. Query strains that harbor a chromosomal Ty1 his3AI reporter element and either the rtt101Δ or med1Δ mutation, both of which confer a hypertransposition phenotype, were mated to 4,847 haploid ORF deletion strains. Retrotransposition was measured in the double mutant progeny, and a set of 275 ORF deletions that suppress the hypertransposition phenotypes of both rtt101Δ and med1Δ were identified. The corresponding set of 275 retrotransposition host factors (RHFs) includes 45 previously identified Ty1 or Ty3 co-factors. More than half of the RHf genes have statistically robust human homologs (E < 1 x 10^-10). The level of unintegrated Ty1 cDNA in 181 rhfΔ single mutants was altered <2-fold, suggesting that the corresponding co-factors stimulate retrotransposition at a step after cDNA synthesis. However, deletion of 43 RHf genes, including specific ribosomal protein and ribosome biogenesis genes and RNA degradation, modification and transport genes resulted in low Ty1 cDNA levels. The level of Ty1 Gag but not RNA was reduced in ribosome biogenesis mutants bud21Δ, hcr1Δ, loc1Δ, and puf6Δ.

Conclusion: Ty1 retrotransposition is dependent on multiple co-factors acting at different steps in the replication cycle. Human orthologs of these RHfs are potential, or in a few cases, presumptive HIV-1 co-factors in human cells. RHf genes whose absence results in decreased Ty1 cDNA include characterized RNA metabolism and modification genes, consistent with their having roles in early steps in retrotransposition such as expression, nuclear export, translation, localization, or packaging of Ty1 RNA. Our results suggest that Bud21, Hcr1, Loc1, and Puf6 promote efficient synthesis or stability of Ty1 Gag.

Keywords: Ty1, LTR-retrotransposon, Saccharomyces cerevisiae, Host factor, Ribosome, Biogenesis

Background

Reverse transcription of RNA generates a significant portion of the eukaryotic genome, including retrotransposons, endogenous retroviruses, retrogene, processed pseudogenes, and other retrosequences [1,2]. The reverse transcriptases that create retrosequences are encoded by retrotransposons. To understand how eukaryotic hosts harness retrotransposons to create adaptive genome rearrangements and novel genes and regulatory sequences, it is essential to identify host factors that are co-opted for retrotransposon mobility and elucidate their mechanism of action.

Three classes of eukaryotic retrotransposons have been described: LTR (long terminal repeat)-retrotransposons, TP (target-primed)-retrotransposons, and Y (tyrosine-recombinase)-retrotransposons [3]. LTR-retrotransposons, which are structurally and functionally related to infectious retroviruses, are the only transposable elements in eukaryotic genomes.
the nuclear genome of the budding yeast, *Saccharomyces cerevisiae*. Ty1 elements comprise the most abundant, highly expressed and mobile of the LTR-retrotransposon families in the *S. cerevisiae* genome. Ty1 elements consist of direct terminal repeats flanking two overlapping open reading frames, *gag* (*TYA1*) and *pol* (*TYB1*). The Ty1 mRNA, which is transcribed by RNA polymerase II, capped and polyadenylated, is the template for translation of all Ty1 proteins as well as for reverse transcription of the full-length cDNA. Two primary translation products are synthesized: p49-Gag and p199-Gag-Pol, the latter resulting from a programmed ribosomal frameshift from *gag* to *pol*. Ty1 mRNA is encapsulated into cytoplasmic virus-like particles (VLPs) consisting of Ty1 Gag and Gag-Pol. Inside the VLP, Gag is processed to its mature form (p45-Gag), while Gag-Pol is processed into p45-Gag, protease (PR), integrase (IN), and reverse transcriptase/RNaseH (RT/RH). In mature VLPs, Ty1 RNA is reverse-transcribed into a linear, double-stranded cDNA. The cDNA, in association with IN, is then transported back to the nucleus, where it is integrated into chromosomal DNA [4,5]. Alternatively, Ty1 cDNA can enter the genome by recombination at chromosome break sites [6].

Although the majority of the 30 to 35 Ty1 elements in the genome of *S. cerevisiae* laboratory strains are functional for retrotransposition, and Ty1 RNA is one of the most abundant mRNAs in the cell, there is only one retrotransposition event per 10,000 cells approximately [7-9]. The low frequency of endogenous Ty1 element mobility presents a significant barrier to performing genetic screens for host co-factors that facilitate retrotransposition. The first genetic screen for Ty1 retrotransposition host factors (RHF s) overcame this barrier by using a plasmid-based Ty1 element expressed from the inducible GAL1 promoter (pGTY1). This screen identified 99 non-essential RHF genes that promote pGTy1HIS3 retrotransposition [10]. However, pGTy1 expression has been shown to override host-mediated transpositional dormancy and copy number control, and therefore it could mask the hypotransposition phenotype of many Ty1 co-factor mutants [11-13]. A recent screen employed an integrating plasmid-based Ty1 element expressed from the native promoter and tagged with the retrotransposition indicator gene, *his3AI*. This screen identified 168 non-essential genes as RHFs [14]; however, there was little overlap between the sets of candidate RHFs identified in these two screens, and relatively few of these RHFs have been characterized. Two similar screens for co-factors of the distantly related Ty3 LTR-retrotransposon using a low copy number or high copy number pGTY3 element identified 21 and 66 Ty3 co-factors, respectively, including a few that are also necessary for Ty1 retrotransposition [15-17].

Aside from RHFs that are required for Ty1 transcription (reviewed in [4,5]), several RHFs that promote post-transcriptional steps in retrotransposition of endogenous Ty1 elements have been characterized. Dbr1, an intron RNA lariat debranching enzyme, acts at a post-translational step to stimulate Ty1 cDNA accumulation by a thoroughly investigated but elusive mechanism [18-21]. The mRNA decapping complex, Dcp1-Dcp2, the 5’ to 3’ mRNA exonuclease, Xrn1, and components of the deadenylation-dependent mRNA decay pathway (Dhh1, Lsm1, Pat1, and Ccr4) and the nonsense-mediated mRNA decay pathway (Upf1, Upf2, and Upf3) stimulate post-translational steps in retrotransposition [22-24]. The 5’ to 3’ mRNA decay pathways are thought to regulate degradation of a Ty1 antisense transcript that interferes with transposition and to facilitate packaging of Ty1 RNA into VLPs [12,23,24]. Bud22 is a ribosome biogenesis factor required for 40 S ribosomal subunit formation. In a bud22Δ mutant, the levels of Ty1 Gag, especially the processed p45-Gag, and VLPs are decreased, and translational frameshifting from *gag* to *pol* is reduced [14]. Hos2 and Set3, components of the SET3 histone deacetylase complex, promote integration of Ty1 cDNA [25].

The goal of this study was to identify a more complete set of RHFs that promote retromobility of endogenous chromosomal Ty1 elements. A chromosomal Ty1 element marked with *his3AI* gives rise to marked Ty1HIS3 retrotransposition events in one in approximately 10⁷ cells [7]. To identify host co-factors that are necessary for these rare events, we used an iterative synthetic genetic array (SGA) approach. This method involved screening the non-essential ORF deletion collection for gene deletions that suppress the hypertransposition phenotypes of two different mutants. One of the hypertransposition mutants carried a deletion of *RTT101*, a gene encoding the cullin-component of an E3 ubiquitin ligase. Rtt101 functions in DNA replication fork protection and non-functional rRNA decay [26]. The second was a deletion of *MEDI*, which encodes a non-essential subunit of the RNA polymerase II mediator complex involved in transcriptional regulation [27]. Ty1 retrotransposition and cDNA are increased post-transcriptionally in both *rtt101Δ* and *med1Δ* mutants, but by different mechanisms [28,29]. The DNA damage checkpoint pathway is essential for the hypertransposition phenotype of an *rtt101Δ* mutant, whereas deletion of genes encoding components of the DNA damage checkpoint pathway has no effect on hypertransposition in a *med1Δ* mutant. Because the hypertransposition phenotypes result from perturbation of distinct pathways, we reasoned that genes whose deletion suppresses hypertransposition in both *rtt101Δ* and *med1Δ* mutants would encode general activators of retrotransposition. Here we describe the identification of 275 candidate Ty1 RHFs. Forty-five were previously identified as Ty1 or Ty3 co-factors in...
Figure 1 (See legend on next page.)
small or high-throughput genetic screens, providing verification of the RHFs identified by the iterative SGA approach. Moreover, 43 rhfΔ mutations result in low Ty1 cDNA levels in the absence of either query mutation, indicating that the corresponding RHFs function during or prior to cDNA accumulation. Genes involved in ribosome biogenesis were enriched in the entire set of 275 RHFs and in the subset with retrotransposition category (Figure 3A). Schematic of the Ty1his3AI retrotransposition assay on one SC-His plate of rtt101Δ LEU2Δ::kanMX progeny. Cells that sustained a retrotransposition event give rise to His+ papillae, which were counted at each address. Addresses that are blank lack progeny because of synthetic lethality or slow growth (green circles). Addresses with S5 His+ papillae (red circles) harbor progeny with reduced retrotransposition. The parental rtt101Δ strain (blue circle) was plated in an empty address prior to induction of retrotransposition.

Results

Iterative synthetic genetic array screen for RHF genes
To identify co-factors required for Ty1 retrotransposition, we designed a genetic screen using a modification of the SGA protocol [30,31]. First, we constructed a strain carrying a single chromosomal Ty1his3AI element adjacent to a selectable marker (Figure 1A). Insertion of the retrotransposition indicator gene his3AI into a chromosomal Ty1 element allows cells in which this marked element undergoes retrotransposition to be detected as His+ prototrophs [7]. Strain Y9230, which carries a can1Δ::Ste2p-LIRA3 allele for selection of haploid MATa progeny [31], was modified by introducing his3AI into the 3′ untranslated region of YJRWTy1-1,2, and the MET15 marker downstream of YJRWTy1-2. Subsequently, the rtt101Δ::LEU2 or med1Δ::LEU2 mutation was introduced into the strain to generate two query strains with elevated levels of Ty1 retromobility. Each query strain was mated to the constituents of the haploid non-essential ORF deletion library (Figure 1B). Diplod strains were sporulated, and aliquots of the spore cultures transferred to a series of selective media plates to obtain haploid MATa progeny that contained the query deletion (rrt101Δ or med1Δ), the Ty1his3AI-MET15 allele, and an orfΔ::KanMX allele. Haploid progeny of each query strain were subjected to a quantitative assay for Ty1his3AI retrotransposition. The haploid strains were grown in YPD broth at 20°, a temperature that is permissive for retrotransposition. An aliquot of each culture was spotted onto YPD agar containing G418 and onto SC-His agar. At each address where haploid progeny grew as a confluent patch on YPD agar with G418, the number of His+ papillae was determined as a measure of the frequency of Ty1his3AI retrotransposition (Figure 1C).

To ascertain whether our selection protocol yielded progeny that were haploid, we tested 78 Leu+ Ura+ Met+ Can+ G418R progeny strains derived from the rtt101Δ query strain for sensitivity to 0.05% methylmethanesulfonate (MMS), which is conferred by the recessive rtt101Δ mutation. All 78 strains were MMS (data not shown), indicating that they were haploid.

A pilot experiment was performed to determine whether the retrotransposition phenotype of progeny strains obtained by SGA selection was reproducible. One plate of 94 yeast orfΔ::kanMX strains was mated to the rtt101Δ query strain, sporulation was induced, and independent haploid progeny were selected 10 times. All 94 rtt101Δ orfΔ::kanMX progeny strains were viable in all 10 trials. The parental rtt101Δ strain, which was grown in an empty address on each of the 10 plates of progeny, yielded an average of 25 ± 3 His+ papillae per trial. Each trial with haploid progeny at each address was assigned to a binary class depending on whether or not there was a ≥5-fold reduction in His+ papillae relative to the average for the rtt101Δ strain. We determined the fraction of trials at each address that fell into the ≥5-fold reduced retrotransposition category or <5-fold reduced category (Figure 2). At 84 of the 94 (89%) addresses, retrotransposition was reduced ≥5-fold in eight or more of the 10 trials (Figure 2, yellow bars) or in two to zero trials (Figure 2, red bars). Only 10 of the 94 addresses had fewer than eight trials in one category or the other (Figure 2, blue bars). Thus, the results of the retrotransposition assay
in independently derived progeny of the same genotype were highly reproducible.

The protocol was applied genome-wide by mating rtt101Δ and med1Δ query strains to 4,847 haploid ORF deletion strains. Following sporulation, independent haploid progeny were selected twice from spores derived from each query strain. Both sets of progeny from each query strain were tested to determine the retrotransposition frequency. When mated to the query strain were tested to determine the retrotransposition frequency. When mated to the query strain, 4,847 of the ORF deletion strains yielded viable haploid progeny in both trials. Of these, 1,419 strains had ≥5-fold reduction in His+ papillae formation relative to the Δ query strain, representing a 5-fold reduction in retrotransposition. Using the med1Δ query strain, 4,289 of the ORF deletion strains yielded viable progeny in both trials. The parental med1Δ query strain had an average of 14.0 ± 0.6 His+ papillae in each trial. Since the parental rtt101Δ query strain was isolated 10 independent times, and retrotransposition was measured in all 940 isolates. The fraction of trials at each address that yielded a ≥5-fold reduction in His+ papillae formation relative to the rtt101Δ query strain is plotted on the x-axis. The percentage of addresses within each category is plotted on the y-axis.

275 RHFs identified in overlapping screen sets

Of the 275 RHFs identified by SGA analysis, 45 (16%) were identified previously as Ty1 or Ty3 retrotransposition co-factors (Table 1 [10,14-16]). Of these, 26 were found in a screen for activators of an integrating plasmid-based Ty1his3AI element [14]. The fact that single mutants lacking these 45 co-factors are defective for retromobility of plasmid-based Ty1 or Ty3 elements provides confirmation that the modified SGA screen successfully identified bona fide Ty1 co-factors.

The 275 candidate RHFs include 190 (69%) that have statistically significant human homologs (E-value score of <0.01; see Additional file 1), and 149 (54%) that have E-value scores of <1 x 10-10, suggesting evolutionary and potentially functional conservation. Twenty-one of the 275 RHFs are encoded by misidentified or dubious ORFs. Many of these ORFs partially overlap characterized genes, which could play a role in retrotransposition; however, the effects of overlapping ORFs on retrotransposition have not been investigated further.

To explore the cellular role of RHFs, we used GO Slim Mapper to assign the RHF genes to gene ontology categories based on molecular function and biological process (see additional file 2). A histogram showing the distribution of (a) suppressors of rtt101Δ hypertransposition, (b) suppressors of med1Δ hypertransposition, and (c) RHF genes (that is, genes in the intersecting set of suppressors) compared to the distribution of (d) all yeast genes in GO functional categories is shown in Figure 3. The rtt101Δ suppressors and med1Δ suppressors were distributed among all GO functional categories and the frequencies of distribution were similar in most categories, which suggests that both screens were biased toward general activators of retrotransposition rather than rtt101Δ- or med1Δ-specific suppressors. In a small number of categories, notably lipid-binding genes, the frequencies of rtt101Δ suppressors and med1Δ suppressors were equivalent, but there was little or no overlap between the sets of genes identified, resulting in a low frequency of RHF genes in the category. However, RHF genes were found in most GO functional categories. In a small number of categories, the frequency of RHF genes is substantially higher (for example, structural constituent of ribosome) or lower (for example, rRNA binding) compared to the genome-wide frequency, but most functional categories have similar frequencies of RHF genes and all genes. Overall, the data reveal broad distribution of RHF genes among functional gene categories, which is likely to reflect the fact that host factors are required for many steps of Ty1 retrotransposition.

We used FunSpec (http://funspec.med.utoronto.ca) to determine whether our set of RHFs was significantly enriched for any of 459 MIPS functional categories and found that ribosomal proteins were enriched (P = 7.39 x 10^-06). The screen identified 26 of 246
| RHF  | Systematic gene name | Cellular function                                                                                                                                                                                                 | Reference | Relative Ty1 cDNA level (rhfΔ/RHF) |
|------|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|-----------------------------------|
| Apq12| YIL040W              | Protein required for nuclear envelope morphology, nuclear pore complex localization, mRNA export from the nucleus; exhibits synthetic lethal genetic interactions with genes involved in lipid metabolism                                    | [14]      | 1.39                              |
| Bro1 | YPL084W              | Class E vacuolar protein sorting factor that coordinates deubiquitination in the multivesicular body (MVB) pathway                                                                                                                                                  | [16]      | 1.54                              |
| Ccr4 | YAL021C              | Component of the CCR4-NOT transcriptional complex, which is involved in regulation of gene expression; component of the major cytoplasmic deaenylylase, which is involved in mRNA poly(A) tail shortening                                | [23]      | 0.65                              |
| Cdc50| YCR094W              | Endosomal protein that interacts with phospholipid flipase Dns2p; interaction with Cdc50p is essential for Dns2p catalytic activity                                                                                                                                 | [14]      | 0.38                              |
| Cpr7 | YJR032W              | Peptidyl-prolyl cis-trans isomerase, Hsp90 co-chaperone                                                                                                                                                                                                               | [10,16]   | 0.67                              |
| Dhh1 | YDL160C              | Cytoplasmic DExD/H-box helicase, stimulates mRNA decapping                                                                                                                                                                                                           | [14,16]   | 0.23                              |
| Elp2 | YGR200C              | Subunit of transcriptional elongator complex (HAT)                                                                                                                                                                                                                   | [10]      | 0.21                              |
| Glc2 | YDR272W              | Cytoplasmic glyoxalase II, catalyzes the hydrolysis of S-D-lactoylglutathione into glutathione and D-lactate                                                                                                                                                        | [14]      | 1.04                              |
| Hda3 | YPR179C              | Subunit of a possibly tetrameric trichostatin A-sensitive class II histone deacetylase complex that contains an Hda1p homodimer and an Hda2p-Hda3p heterodimer; required for the activity of the complex                           | [14]      | 1.84                              |
| Hmo1 | YDR174W              | Chromatin-associated high mobility group (HMG) family member involved in genome maintenance; rDNA-binding component of the Pol I transcription system; associates with a 5'-3' DNA helicase and Fpr1p, a poly(rI) polymerase | [14]      | 0.19                              |
| Ksp1 | YHR082C              | Nuclear serine/threonine kinase; stress response                                                                                                                                                                                                                   | [15]      | 1.79                              |
| Lsc1 | YFR001W              | Nuclear protein involved in asymmetric localization of ASH1 mRNA; binds double-stranded RNA in vitro; constituent of 66S pre-ribosomal particles                                                                                                     | [14]      | 0.14                              |
| Lsm1 | YJL124C              | Component of heterohexameric complex involved in cytoplasmic mRNA degradation                                                                                                                                                                                         | [10,14]   | 0.53                              |
| Met5 | YJR137C              | Sulfite reductase, involved in amino acid biosynthesis and transcription repressed by methionine                                                                                                                                                                     | [15]      | 1.04                              |
| Mig3 | YER028C              | Probable transcriptional repressor involved in response to toxic agents that inhibit ribonucleotide reductase; phosphorylation by Snf1p or the Mec1p pathway inactivates Mig3p, allowing induction of damage response genes                               | [14]      | 1.75                              |
| Ncl1 | YBL024W              | tRNA methyltransferase                                                                                                                                                                                                                                               | [15]      | 0.49                              |
| Nlp100| YPL174C              | Large subunit of the dynactin complex, which is involved in partitioning the mitotic spindle between mother and daughter cells; putative ortholog of mammalian p150 (glued)                                                                 | [14]      | 1.06                              |
| Nup133| YKR082W              | Subunit of the Nup84p subcomplex of the nuclear pore complex                                                                                                                                                                                                        | [10]      | 3.13                              |
| Nup170| YBL079W              | Subunit of the nuclear pore complex (NPC), required for NPC localization of specific nucleoporins; involved in nuclear envelope permeability and chromosome segregation; has similar to Nup157; essential role, with Nup157, in NPC assembly | [14]      | 0.66                              |
| Oca4 | YCR005C              | Cytoplasmic protein required for replication of Brome mosaic virus in S. cerevisiae                                                                                                                                                                                      | [14]      | 0.43                              |
| Pde2 | YOR360C              | High-affinity cyclic AMP phosphodiesterase, component of the cAMP-dependent protein kinase signaling system, protects the cell from extracellular cAMP                                                                                                                                 | [14]      | 0.92                              |
| Ref2 | YDR195W              | RNA-binding protein involved in the cleavage step of mRNA 3'-end formation prior to polyadenylation, and in snoRNA maturation; part of holo-CPP subcomplex APT, which associates with 3'-ends of snoRNA- and mRNA-encoding genes | [14]      | 0.17                              |
| Rpl16B| YNL069C              | Component of the large (60 S) ribosomal subunit; binds to 5.8 S rRNA; has similarity to Rpl16A                                                                                                                                                                         | [10]      | 0.51                              |
| ORF   | Description                                                                                                                                  | Ref | Score |
|-------|----------------------------------------------------------------------------------------------------------------------------------------------|-----|-------|
| Rpl27a | Protein component of the large (60 S) ribosomal subunit, nearly identical to Rpl27B                                                          | [14] | 0.19  |
| Rpp1A  | Ribosomal stalk protein P1 alpha, involved in the interaction between translational elongation factors and the ribosome                           | [10] | 1.23  |
| Rps19b | Protein component of the small (40 S) ribosomal subunit, required for assembly and maturation of pre-40 S particles; mutations in human RPS19 are associated with Diamond Blackfan anemia; nearly identical to Rps19A | [14] | 0.28  |
| Rps25a | Protein component of the small (40 S) ribosomal subunit; nearly identical to Rps25B                                                            | [14] | 0.19  |
| Ski8   | Ski complex component and WD-repeat protein, mediates 3′-5′ RNA degradation by the cytoplasmic exosome; also required for meiotic double-strand break recombination | [14] | 0.58  |
| Snf5   | Subunit of the SWI/SNF chromatin remodeling complex involved in transcriptional regulation; functions interdependently in transcriptional activation with Snf2p and Snf6p | [32] | 0.09  |
| Snf6   | Subunit of the SWI/SNF chromatin remodeling complex involved in transcriptional regulation; functions interdependently in transcriptional activation with Snf2p and Snf6p | [32] | 0.27  |
| Spt3   | Subunit of the SAGA and SAGA-like transcriptional regulatory complexes, interacts with Spt15p to activate transcription of some RNA polymerase II-dependent genes | [33] | 0.09  |
| Spt8   | Subunit of the SAGA transcriptional regulatory complex                                                                                       | [14] | 0.10  |
| Spt10  | Putative histone acetylase; sequence-specific activator of histone genes                                                                      | [10,14] | 1.17  |
| Ssp1   | Stimulates the ATPase and helicase activities of Prp43p; acts with Prp43p to stimulate 18 s rRNA maturation by No1p; component of pre-ribosomal particles | [14] | 0.63  |
| Sse1   | ATPase; Hsp90 co-chaperone; binds unfolded proteins; member of the heat shock protein 70 (HSP70) family                                         | [10] | 0.25  |
| Swi3   | Subunit of the SWI/SNF chromatin remodeling complex                                                                                         | [14] | 0.44  |
| Tgs1   | Trimethyl guanosine synthase, conserved nucleolar methyl transferase that converts the m(7)G cap structure of snRNAs, snoRNAs, and telomerase TLC1 RNA to m(2,2,7)G; also required for ribosome synthesis and nucleolar morphology | [14] | 1.44  |
| Thrp2  | Subunit of the THO/TREX complex, couples transcription to mRNA export                                                                         | [10] | 0.98  |
| Trk1   | Component of the Trk1p-Trk2p high-affinity potassium transport system; plasma membrane protein                                                | [10,14] | 1.14  |
| Ump1   | Chaperone required for correct maturation of the 20 S proteasome                                                                             | [14] | 0.36  |
| Upf1   | ATP-dependent RNA helicase of the SF1 superfamily involved in nonsense-mediated mRNA decay (NMD); required for efficient translation termination at nonsense codons and targeting of NMD substrates to P-bodies; involved in telomere maintenance | [14,23] | 0.25  |
| Upf3   | Component of the NMD pathway, along with Nam7 and Nmd2/Upf2; involved in decay of mRNA containing nonsense codons                               | [14,23] | 0.29  |
| Vma16  | Subunit c of vacuolar-ATPase, which functions in acidification of the vacuole; one of three proteolipid subunits of the V<sup>o</sup> domain         | [15] | 1.32  |
| Vph1   | Subunit a of vacuolar-ATPase, V<sup>o</sup> domain which functions in acidification of the vacuole; one of three proteolipid subunits of the V<sup>o</sup> domain | [10,16] | 1.55  |
| YML009C-A | Dubious ORF unlikely to encode a functional protein, based on available experimental and comparative sequence data; ORF overlaps the essential gene, SPT5 | [14] | N.D.  |
ribsosomal proteins, including the large ribosomal subunit constituents Rpl7a, Rpl16b, Rpl19a, Rpl27a, Rpl31a, Rpl33b, Rpl34a, Rpl37a, and Rpl43a, small ribosomal subunit components Rps11a, Rps19a, Rps19b, Rps25a, Rps27b, and Rps30a, ribosomal stalk protein Rpp1a, ribosome biogenesis factors Rsa3 and Dpb7, translation initiation factor eIF2A (encoded by \textit{YGR054W}), and mitochondrial ribosomal subunits Mrpl7, Mrpl8, Mrpl39, Mrpl49, Mrps28, and Mrp17. The final protein identified was Met13, which is erroneously classified as a mitochondrial ribosomal protein. In addition to ribosomal proteins identified by FunSpec, seven additional ribosome biogenesis factors (Bud21, Hcr1, Loc1, Mrt4, Rkm4, Sq51, and Utp30) and a ribosome-associated protein chaperone (Zuo1), were identified. Thus, 33 of the 275 RHFs (12%) are constituents of the ribosome or required for ribosome biogenesis.

**Stringency of iterative SGA screen**
Deletion strains that did not yield viable progeny in all four trials, or whose progeny did not show a ≥5-fold reduction in Ty1 \textit{his3AI} retromobility in all four trials were not identified as \textit{rhfΔ} mutants. Thus, some Ty1 co-factor mutants may not have been found by iterative SGA analysis because of synthetic lethality under transposition-induction conditions or because their absence did not strongly suppress hypertransposition in both the \textit{med1Δ} and the \textit{rtt101Δ} mutants. To understand the limitations of the screen, we examined the results for eight previously characterized Ty1 co-factor genes that were not successfully identified here as \textit{RHF} genes. Seven of eight known Ty1 co-factor mutants were not identified because the mutation failed to suppress retrotransposition in one or both trials of either the \textit{rtt101Δ} screen or the \textit{med1Δ} screen. The co-factor gene deletion \textit{bud22Δ} failed to suppress \textit{rtt101Δ} hypertransposition in either trial, while \textit{tec1Δ} did not suppress \textit{rtt101Δ} hypertransposition in one trial. On the other hand, retrotransposition-defective \textit{xrn1Δ}, \textit{hos2Δ}, \textit{set3Δ}, \textit{pat1Δ}, and \textit{upf2Δ} mutations failed to suppress \textit{med1Δ} hypertransposition in one or both (\textit{hos2Δ}) trials. The eighth Ty1 co-factor mutant, \textit{dbi1Δ}, was not identified.
| Retrotransposition host factor | Systematic ORF name | Cellular function | Relative Ty1 cDNA level (rhfΔ/RHF) | Genomic DNA samples analyzed (n) |
|--------------------------------|---------------------|-------------------|-----------------------------------|---------------------------------|
| Afr1                          | YDR085C             | Protein required for pheromone-induced projection (shmoo) formation; regulates septin architecture during mating; has an RXFX motif that mediates targeting of Glc7 to mating projections; interacts with Cdc12 | 0.42                            | 2                               |
| Atp17                         | YDR377W             | Subunit f of the F0 sector of mitochondrial F1F0 ATP synthase, which is a large, evolutionarily conserved enzyme complex required for ATP synthesis | 0.34                            | 2                               |
| Bud21                         | YOR078W             | Also known as UTP16; component of small ribosomal subunit (SSU) processome that contains U3 snoRNA | 0.29                            | 2                               |
| Cdc50                         | YCR094W             | Endosomal protein that interacts with phospholipid flipase Ds2; interaction with Cdc50p is essential for Ds2 catalytic activity; mutations affect cell polarity and polarized growth | 0.38                            | 2                               |
| Cth1                          | YDR151C             | Member of the CCCH-zinc finger family; has similarity to mammalian Tis11 protein, which activates transcription and also has a role in mRNA degradation; may function with Tis11 in iron homeostasis | 0.30                            | 2                               |
| Dbf20                         | YPR1111W            | Ser/Thr kinase involved in late nuclear division, one of the mitotic exit network (MEN) proteins; necessary for the execution of cytokinesis; ortholog of human NDR2 kinase | 0.45                            | 3                               |
| Dhp7                          | YKR024C             | Putative ATP-dependent RNA helicase of the DEAD-box family involved in ribosomal biogenesis | <0.01                           | 2                               |
| Dfg10                         | YLO499W             | Probable polypropen reductase that catalyzes conversion of polypropen to dolichol, the precursor for N-glycosylation; mutations in ortholog SRD1A3 confer CDG1Q (Congenital Disorders of Glycosylation type 1Q) | 0.27                            | 2                               |
| Dgr2                          | YKL121W             | Protein of unknown function; null mutant is resistant to 2-deoxy-D-glucose | 0.32                            | 3                               |
| Dhh1                          | YDL160C             | Cytoplasmic DExD/H-box helicase, stimulates mRNA decapping, coordinates distinct steps in mRNA function and decay, interacts with both the decapping and deadenylyase complexes; ortholog of the human oncogene DDX6/p54/RCK | 0.23                            | 5                               |
| Elp2                          | YGR200C             | Subunit of elongator complex, which is a component of the RNA polymerase holoenzyme and required for modification of wobble uridines in tRNA; ortholog of human ELP2/STATIP1 gene | 0.21                            | 2                               |
| Hcr1                          | YLR192C             | Dual function protein involved in translation initiation as a substoichiometric component (eif3j) of translation initiation factor 3 (eIF3) and required for processing of 20 S pre-rRNA; ortholog of human EIF3J gene | 0.29                            | 3                               |
| Hit1                          | YJR055W             | Unknown function, required for growth at high temperature | 0.24                            | 2                               |
| Hmo1                          | YDR174W             | Chromatin associated high mobility group (HMG) family member involved in genome maintenance; rDNA-binding component of the Pol I transcription system; associates with a 5′-3′ DNA helicase and Fpr1, a prolyl isomerase | 0.19                            | 3                               |
| Kgd1                          | YIL125W             | Component of the mitochondrial alpha-ketoglutarate dehydrogenase complex, which catalyzes a key step in the tricarboxylic acid (TCA) cycle, the oxidative decarboxylation of alpha-ketoglutarate to form succinyl-CoA; ortholog of human OGDHL gene | 0.27                            | 2                               |
| Loc1                          | YFR001W             | Nuclear protein involved in asymmetric localization of ASH1 mRNA; binds double-stranded RNA in vitro; co-localizes with large subunit precursor of ribosome | 0.14                            | 3                               |
| Los1                          | YKL205W             | Nuclear pore protein involved in nuclear export of pre-rRNA and in re-export of mature tRNAs after retrograde import from the cytoplasm; ortholog of human exportin-T gene, XPOT | 0.44                            | 3                               |
| Lst7 | YGR057C | Protein possibly involved in a post-Golgi secretory pathway; required for the transport of nitrogen-regulated amino acid permease Gap1 from the Golgi to the cell surface | 0.44 | 4 |
| Mrt4 | YKL009W | Protein involved in mRNA turnover and large ribosome assembly, co-localizes with large subunit precursor of ribosome; ortholog of human MRT04 gene | 0.17 | 2 |
| Ncl1 | YBL024W | S-adenosyl-L-methionine-dependent tRNA: m5C-methyltransferase, methylates cytosine to m5C at several positions in tRNAs and intron-containing pre-tRNAs; similar to Nop2 and human proliferation associated nucleolar protein p120 | 0.49 | 4 |
| Oca4 | YCR095C | Cytoplasmic protein required for replication of Brome mosaic virus in S. cerevisiae, which is a model system for studying replication of positive-strand RNA viruses | 0.43 | 2 |
| Ref2 | YDR195W | RNA-binding protein involved in the cleavage step of mRNA 3’-end formation prior to polyadenylation, and in snRNA maturation; part of holo-cpf subcomplex APT, which associates with 3’-ends of snoRNA- and mRNA-encoding genes | 0.17 | 2 |
| Rkm4 | YDR257C | Ribosomal lysine methyltransferase specific for monomethylation of Rpl42a and Rpl42b (lysine 55); nuclear SET-domain containing protein | 0.41 | 4 |
| Rpl7a | YGL076C | Protein component of the large (60 S) ribosomal subunit, nearly identical to Rpl7b; ortholog of human L7 ribosomal protein gene | 0.15 | 4 |
| Rpl19a | YBR084C-A | Protein component of the large (60 S) ribosomal subunit, nearly identical to Rpl19b; ortholog of human L19 ribosomal protein gene | 0.24 | 2 |
| Rpl27a | YGL076C | Protein component of the large (60 S) ribosomal subunit; nearly identical to Rpl27b; ortholog of human L27 ribosomal protein gene | 0.19 | 2 |
| Rpl31a | YDL075W | Protein component of the large (60 S) ribosomal subunit, nearly identical to Rpl31b; ortholog of human L31 ribosomal protein gene | 0.10 | 2 |
| Rpl43a | YPR043W | Protein component of the large (60 S) ribosomal subunit, identical to Rpl43b; ortholog of human ribosomal protein L37 gene | 0.15 | 3 |
| Rps19b | YNL302C | Protein component of the small (40 S) ribosomal subunit, required for assembly and maturation of pre-40 S particles; mutations in human RPS19 are associated with Diamond Blackfan anemia; nearly identical to Rps19a | 0.28 | 4 |
| Rps25a | YGR027C | Protein component of the small (40 S) ribosomal subunit; nearly identical to Rps25b; ortholog of human S25 ribosomal protein gene | 0.19 | 2 |
| Rps30a | YLR287C-A | Protein component of the small (40 S) ribosomal subunit; nearly identical to Rps30b; ortholog of human S30 ribosomal protein | 0.20 | 2 |
| Snf5 | YBR289W | Subunit of the SWI/SNF chromatin remodeling complex involved in transcriptional regulation; functions interdependently in transcriptional activation with Snf2 and Snf6 | 0.09 | 2 |
| Snf6 | YHL025W | Subunit of the SWI/SNF chromatin remodeling complex involved in transcriptional regulation; functions interdependently in transcriptional activation with Snf2 and Snf5 | 0.27 | 2 |
| Snt1 | YCR033W | Subunit of the Set3C deacetylase complex that interacts directly with the Set3C subunit, Sif2p; putative DNA-binding protein | 0.21 | 2 |
| Spf1 | YEL031W | P-type ATPase, ion transporter of the ER membrane involved in ER function and Ca2+ homeostasis; required for regulating Hmg2 degradation | 0.42 | 2 |
| Spt3 | YDR392W | Subunit of the SAGA and SAGA-like transcriptional regulatory complexes, interacts with Spt15 to activate transcription of some RNA polymerase II-dependent genes; also inhibits transcription at some promoters | 0.09 | 3 |
| Gene   | Accession | Function and Other Information                                                                 | p-value | Fold |
|--------|-----------|-----------------------------------------------------------------------------------------------|---------|------|
| Spt8   | YLR055C   | Subunit of the SAGA transcriptional regulatory complex but not present in SAGA-like complex SLIK/SALSA; required for SAGA-mediated inhibition at some promoters | 0.10    | 2    |
| Sse1   | YPL106C   | ATPase that is a component of the heat shock protein Hsp90 chaperone complex; binds unfolded proteins; member of the HSP70 family | 0.25    | 2    |
| Swi3   | YJL176C   | Subunit of the SWI/SNF chromatin remodeling complex                                               | 0.44    | 3    |
| Ump1   | YBR173C   | Short-lived chaperone required for correct maturation of the 20 S proteasome; may inhibit premature dimerization of proteasome half-mers; degraded by proteasome upon completion of its assembly | 0.36    | 3    |
| Upf1   | YMR080C   | Also known as Nam7; ATP-dependent RNA helicase of the SF1 superfamily involved in nonsense mediated mRNA decay; required for efficient translation termination at nonsense codons and targeting of NMD substrates to P-bodies; involved in telomere maintenance | 0.25    | 4    |
| Upf3   | YGR072W   | Component of the nonsense-mediated mRNA decay (NMD) pathway, along with Upf1 and Upf2; involved in decay of mRNA containing nonsense codons and telomere maintenance; ortholog of human UPF3A and UPF3B genes | 0.29    | 4    |
| YDL124W| YDL124W   | NADPH-dependent alpha-keto amide reductase                                                        | 0.36    | 2    |
because the mutant did not yield viable progeny in one trial with the \( \text{rtt101} \Delta \) query strain. In summary, these results suggest that the set of 275 RHFs is not complete, and that the stringency of the SGA screen was a significant limitation to identifying a complete set of non-essential Ty1 co-factors.

**Forty-three RHFs are required for synthesis or stability of Ty1 cDNA**

To identify RHFs that act before or during Ty1 cDNA synthesis, we measured the level of unintegrated cDNA produced from endogenous Ty1 elements in \( \text{rhf} \Delta \) single mutants. Ty1 cDNA is measured by a Southern blot assay that compares the level of unintegrated Ty1 cDNA to the level of genomic Ty1 element DNA [34]. One to seven biological replicates of 252 of the 275 \( \text{rhf} \Delta \) mutants were analyzed. (Ty1 cDNA levels were not determined in strains with deletions of dubious or mis-identified ORFs.) Total Ty1 cDNA was reduced to <50% of wild-type levels in 43 of the 275 \( \text{rhf} \Delta \) mutants (16%; Table 2). This reduction in cDNA was observed in the absence of either the \( \text{rtt101} \Delta \) or \( \text{med1} \Delta \) mutation.

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**Figure 4** Levels of Ty1 retromobility, RNA, and Gag: GFP protein in six \( \text{rhf} \Delta \) mutants with defects in ribosome biogenesis. (A) The frequency of His\(^+\) prototroph formation (retrotransposition) in wild-type strain JC3807 (WT) and congenic \( \text{rhf} \Delta \) derivatives harboring a chromosomal Ty1his3AI element. The frequency reported for the \( \text{dbp7} \Delta \) strain is the maximum possible frequency determined as if one His\(^+\) colony had formed in each independent culture tested. Error bars: standard error. (B) Northern blot analyses of Ty1 RNA (top panel) and \( \text{PYK1} \) RNA (bottom panel) in each strain, using \( ^{32} \text{P} \)-labeled riboprobes. The ratio of \( ^{32} \text{P} \)-activity in the Ty1 band to \( ^{32} \text{P} \)-activity in the \( \text{PYK1} \) band was determined by phosphorimaging. Ty1/\( \text{PYK1} \) RNA ratios for each strain normalized to that of the wild-type strain are provided below each lane. (C) The average level of Ty1 RNA in total RNA from three biological replicates of each strain relative to the wild-type strain was determined by qPCR analysis (left panel). The \( \text{spt3} \Delta \) strain is a negative control. The average level of RNA derived from the Ty1(\( \text{gag::GFP} \)) -3566 chromosomal element in total RNA from three biological replicates of the wild type strain and the congenic \( \text{bud21} \Delta \) derivative was measured by qPCR analysis. Error bars: standard error. (D) Western blot analyses of total cell lysate with anti-VLP antibody, which recognizes unprocessed p49-Gag and processed p45-Gag (top panel), and anti-alpha-tubulin antibody (bottom panel) as a loading control. (E) Histogram of the mean Gag:GFP fusion protein activity, as measured by flow cytometry, in \( \text{rhf} \Delta \) strains relative to the wild-type strain. Each strain harbors the chromosomal Ty1(\( \text{gag::GFP} \)) -3566 element. Error bars: standard error.
and independently of the Ty1his3A1 assay. Since Ty1 cDNA is a required intermediate in retrotransposition, these mutants are expected to have lower levels of retrotransposition resulting from the decreased levels of total Ty1 cDNA. Therefore, the results confirm that these 43 RHF genes encode host factors that are required for Ty1 retrotransposition. Indeed, eight were previously characterized mutants with defects in Ty1 RNA expression (swi3Δ, snf5Δ, snf6Δ, puf6Δ, and puf8Δ) or post-translational steps in retrotransposition (dhh1Δ, upf1Δ, and upf3Δ). A further demonstration that rhfΔ mutants with reduced levels of Ty1 cDNA are defective in retrotransposition was obtained by introducing the elp2Δ and dfg10Δ mutations into a strain containing Ty1his3A1. The retrotransposition frequency in elp2Δ and dfg10Δ mutants was ≤2% and 3.2 ± 2.0% of the wild-type strain, respectively. Five additional rhfΔ mutants with defects in ribosome biogenesis were also shown to have reduced levels of Ty1his3A1 retrotransposition that are correlated with decreased Ty1 cDNA levels (see below).

Unexpectedly, we also identified 29 RHF genes whose deletion resulted in a ≥2-fold increase in Ty1 cDNA levels (see Additional file 1). In an earlier study, we found that elevated levels of Ty1 cDNA in two of these rhfΔ mutants, ctf4Δ and mms22Δ, are correlated with increased Ty1 retrotransposition [29]; therefore, these two genes were misidentified as RHFs in the SGA analysis. It is not clear why the other 27 rhfΔ mutants have increased levels of cDNA. They could also have been misidentified as rhfΔ mutants, or perhaps cDNA accumulates in these mutants because of defects in nuclear import or integration of cDNA. For example, the nucleoporin Nup133 was identified here and previously as a pGTy1 co-factor [10], yet deletion causes a >3-fold increase in Ty1 cDNA. Deletion of a second component of the Nup84 complex, Nup120, also increased Ty1 cDNA >3-fold (see Additional file 1).

The remaining 181 rhfΔ strains had a <-2-fold increase or decrease in Ty1 cDNA levels. The lack of a substantial decrease in cDNA levels in the absence of these RHFs suggests that these putative co-factors promote a late step in retrotransposition. Twenty-three of the rhfΔ strains with a <2-fold change in cDNA levels were identified as defective in Ty1 and/or Ty3 retrotransposition in previous screens (Table 1), supporting the idea that these candidate RHFs influence Ty1 retrotransposition even though they do not regulate the level of Ty1 cDNA. As a further test of this concept, we deleted a representative gene, Nat4Δ, in a strain carrying a chromosomal Ty1his3A1 element and measured the effect on retromobility. The retrotransposition frequency in the nat4Δ mutant was ≤3% of that of the congenic wild-type strain, even though the level of Ty1 cDNA in a nat4Δ mutant was 101% of that in the wild-type strain. Thus, the histone acetyltransferase Nat4 promotes Ty1 retrotransposition at a step subsequent to Ty1 cDNA accumulation. Together, our results suggest that a large fraction of RHFs influence late steps in retrotransposition.

Six ribosome biogenesis factors promote a post-transcriptional step in Ty1 retrotransposition

The 43 RHFs that are required for efficient Ty1 cDNA accumulation include eight ribosomal protein paralogs, six ribosome biogenesis factors and a regulator of rRNA transcription (Table 2). Thus, translation of Ty1 RNA could be an important level of host contribution to retrotransposition. We explored the possibility that inefficient Ty1 RNA translation results in retrotransposition and cDNA synthesis defects in ribosome biogenesis factor mutants bud21Δ, dhp7Δ, mrt4Δ, loc1Δ, hcr1Δ, and rkm4Δ. We also analyzed another ribosome biogenesis factor mutant, puf6Δ, which we identified in an unrelated study as having reduced Ty1 cDNA levels. The puf6Δ mutant was not found in this screen because med1Δ puf6Δ progeny were not viable, but rtt101Δ puf6Δ progeny had no retrotransposition events. The average Ty1 cDNA level in two biological replicates of the puf6Δ single mutant was 18% of that in a congenic wild-type strain. To confirm that these seven ribosome biogenesis factor genes are required for efficient retrotransposition, each was deleted in strain JC3807, which harbors a chromosomal Ty1his3A1 element. The dhp7Δ mutant had the strongest retrotransposition defect (Figure 4A), consistent with the low levels of Ty1 cDNA in this mutant (Table 2). Retrotransposition was reduced >10-fold in the hcr1Δ, mrt4Δ, and puf6Δ mutants and approximately 4-fold in bud21Δ and loc1Δ mutants. Deletion of the seventh ribosome biogenesis factor gene, RKM4 resulted in very slow growth, and the frequency of retrotransposition in four independent isolates varied more than 10-fold (data not shown). Consequently, the rkm4Δ mutant was not analyzed further.

To determine whether these six rhfΔ mutants with reduced retrotransposition and cDNA levels have a defect in translation of Ty1 RNA, we compared Ty1 RNA and Gag levels in the mutants to those in the wild-type strain. The amount of Ty1 RNA relative to PYK1 RNA in each strain was determined by Northern blot analysis (Figure 4B). Ty1 RNA levels in each mutant were equivalent or increased relative to the wild-type strain, and only the full-length Ty1 transcript was observed. One caveat of this analysis, however, is that the stability of PYK1mRNA could be altered in ribosome biogenesis mutants because of translation defects, resulting in changes in the Ty1/PYK1 RNA ratio that do not result solely from altered Ty1 RNA levels. Therefore, quantitative real-time RT-PCR (qRT-PCR) was performed to measure the level of Ty1 RNA relative to the nuclear non-
coding SNR6 RNA (Figure 4C, left panel). Ty1 RNA levels, as measured by qRT-PCR, were not decreased in the bud21Δ, dbp7Δ, hcr1Δ, loc1Δ, mrt4Δ, or puf6Δ mutant, demonstrating that the retrotransposition defects in these mutants are not a consequence of reduced Ty1 RNA. Moreover, this analysis revealed an 84-fold increase in Ty1 RNA in the dbp7Δ mutant, 3- to 33-fold increases in bud21Δ, hcr1Δ, loc1Δ, and mrt4Δ mutants and no significant change in the puf6Δ mutant. In contrast, an spf3Δ strain, which lacks a critical Ty1 transcription factor, had 14% Ty1 RNA relative to the wild-type strain. Together the data suggest that the ribosome biogenesis factors act at a post-transcriptional step in retrotransposition.

Ty1 Gag expression in the ribosome biogenesis mutants was assayed by Western blotting. As expected, both unprocessed p49-Gag and processed p45-Gag were detected in the wild-type strain (Figure 4D). The p45-Gag/p49-Gag ratio in each of the six mutants was similar to that in the wild-type strain, indicating that the efficiency of Gag processing is not affected in any of the mutants. Total Gag levels appeared to be decreased in the bud21Δ, hcr1Δ, loc1Δ, mrt4Δ, and puf6Δ mutants. To confirm this conclusion using a quantitative method, we used the chromosomal Ty1 translational reporter construct, Ty1(gag::GFP)−3566 in strain JC3807. The reporter consists of a chromosomal Ty1 in which the GFP ORF is fused to the 3’ end of gag at the p45-Gag processing site [35]. The p45-Gag:GFP levels were modestly reduced (44% to 81% of that in the wild-type strain) in bud21Δ, hcr1Δ, loc1Δ, and puf6Δ mutants (Figure 4E). Using qRT-PCR, we confirmed that Ty1(gag::GFP)−3566 RNA was not decreased in a bud21Δ mutant relative to the wild-type strain, so the reduction in p45-Gag:GFP to 44% is not due to Ty1(gag::GFP)−3566 RNA instability (Figure 4C, right panel). Taken together, these data indicate that bud21Δ, hcr1Δ, and loc1Δ have reduced levels of total Ty1 Gag:GFP fusion protein, despite 3- to 33-fold increases in total Ty1 RNA. In addition, the puf6Δ mutant has decreased Gag:GFP levels despite Ty1 RNA levels that are equivalent to the wild-type strain. Our data support the conclusion that Ty1 RNA translation or Gag protein stability is reduced in bud21Δ, hcr1Δ, loc1Δ, and puf6Δ mutants.

The p45-Gag:GFP activity was not significantly changed in the mrt4Δ mutant and slightly increased in the dbp7Δ mutant. While both these strains had significant increases in Ty1 RNA, the data do not allow us to conclude that there is a defect in Gag synthesis or stability. Further analysis will be necessary to determine whether the efficiency of Ty1 RNA translation is altered in dbp7Δ and mrt4Δ mutants.

**Discussion**

The mobility of retrotransposons is tightly regulated by the host cell because of their potential as insertion mutagens and drivers of genome instability. Host-mediated repression of Ty1 mobility presents a significant barrier to identifying co-factors that are required for endogenous Ty1 element retrotransposition. Therefore, we used two independent genetic backgrounds in which endogenous Ty1 element retrotransposition is derepressed to screen for transposition-defective mutants, resulting in the identification of 275 RHF genes. Verification that 45 of the 275 RHFs are bona fide Ty1 co-factors is provided by their previous identification as co-activators of plasmid-based Ty1 or Ty3 elements. We also confirmed that six newly identified RHFs (Bud21, Dpb7, Dgf10, Hcr1, Mrt4, and Nat4) are bona fide Ty1 co-factors by deleting the gene that encodes them in a strain harboring a chromosomal Ty1his3AI element, and demonstrating that retrotransposition is significantly decreased. An additional 18 RHF genes were validated by a >2-fold reduction in Ty1 cDNA when each gene was deleted. Overall, one-quarter of the RHF genes identified here have been validated by independent approaches, suggesting that iterative SGA screening is a powerful strategy for identifying host co-factors of retrotransposition.

The SGA screen for Ty1 co-factors was not exhaustive because only 3,448 (71%) deletion strains yielded progeny that grew well enough for retrotransposition to be measured in both the med1Δ and rtt101Δ trials. Ty1 co-factor gene deletions whose phenotypes were masked by either the rtt101Δ or med1Δ mutation might also have been missed in SGA analysis. Moreover, the requirement that only those gene deletions that reduced retrotransposition ≥5-fold in four separate trials be counted may have precluded the discovery of some bona fide Ty1 co-factors. Indeed, deletion of several previously characterized Ty1 co-factor genes (for example, BUD22, TEC1, XRN1, SET3, PAT1, and UFP2) failed to reduce retrotransposition in both rtt101Δ trials or both med1Δ trials, and thus the genes were not identified as RHF genes. However, the stringency of the screen provides confidence that the RHFs that were identified are necessary for retrotransposition regardless of the genetic background. Although RHFs are not a comprehensive set of Ty1 co-factors, they are broadly distributed among molecular function and biological process categories, suggesting that they affect many different stages of the Ty1 replication cycle or that numerous cellular pathways influence a central process that is necessary for retrotransposition (Figure 3; see Additional file 2).

A few RHF genes, particularly those whose deletion results in extremely elevated Ty1 cDNA levels, may have been misidentified. This group includes MMS2 and CTF4, two characterized Ty1 repressors. Moreover, we assume that POL32, a DNA replication and repair gene whose absence increased Ty1 cDNA more than 30-fold, is a Ty1 repressor, since many other genome maintenance genes...
function as Ty1 repressors [29,36]. Other genes that may have been misidentified as RHFs are those required for efficient splicing, because the intron within the his3AI indicator gene must be removed by splicing in order to be activated. However, there are only a few RHF genes that are known to play a role in RNA splicing (see Additional file 2).

Our study identified many RHF genes that are conserved in eukaryotes. More than half of the RHF genes have statistically robust human homologs, and multiple examples of co-factors with human orthologs were identified (Table 2). Human orthologs of RHF genes could play a role in retroviral replication; indeed, human orthologs of the Ty1 co-factor Dbr1 and a few repressors of Ty1 retrotransposition have been implicated in analogous roles in HIV-1 replication [37–39]. The human ortholog of DBF20, a novel RHF gene that is necessary for Ty1 cDNA accumulation (Table 2), encodes the serine-threonine kinase, NDR2. NDR2 is incorporated into HIV-1 particles and processed by the HIV-1 protease [40]; however, it has not yet been shown to influence HIV-1 replication directly. Two additional RHFs that are necessary for Ty1 cDNA synthesis or stability have human homologs that have been identified in an RNAi screen as presumptive HIV-1 co-factors: Upf3 (homolog of human UPF3B) and Snf1 (homolog of human SNF1LK) [41]. One example of an RHF that could provide a clue to facilitate the characterization of an HIV-1 co-factor is the class E vacuolar protein sorting factor, Bro1. Bro1, which was also identified previously as a Ty3 co-factor, is a homolog of ALIX, which binds to HIV-1 Gag p6 and promotes HIV-1 virion budding [42,43]. Bro1 is also a co-factor for replication of Brome Mosaic Virus (BMV), a positive strand RNA virus that replicates in S. cerevisiae. BMV replication takes place in membrane-bound vesicular invaginations at the perinuclear endoplasmic reticulum [44,45]. Perhaps the fact that Ty1 and Ty3 elements and BMV require Bro1 for replication rather than budding indicates that another function of Bro1 in coordinating the deubiquitination of cargo proteins in multivesicular bodies is important for replication of all these retroelements, including HIV-1 [46].

There is significant overlap between the 275 RHF genes and a set of 97 genes identified in a screen for host genes that affect the replication of BMV in yeast [47]. Twenty genes were identified in both screens ($P = 2.85 \times 10^{-6}$), including 14 genes whose absence inhibited BMV replication or expression (DHHL1, LSM1, LSM6, UMP1, THP2, BRO1, MET13, LGE1, ELF1, SPT8, UFD4, SNF1, SNT1, and OCA4) and six genes that absence increased BMV replication or expression (CDC50, ELP2, SKI8, NIP170, RSC2, and MMS22). This overlap could be a reflection of parallels between BMV replication complex assembly and Ty1 VLP assembly. There are notable similarities between positive-strand RNA virus replication and retroviral particle assembly, including recognition of discrete cis-acting signals in the RNA genome by an element-encoded protein and sequestration of the RNA in a nuclease-resistant, membrane-associated self-assembling protein core [44,45]. Therefore, the finding that Ty1 and BMV utilize an overlapping set of host co-factors may indicate that there is more similarity in the cellular processes that influence replication of positive-sense RNA viruses, retroviruses and retrotransposons than might have been expected based on the differences in their structures and mechanisms of replication.

Ribosome-associated proteins were significantly enriched among RHFs. Many features of Ty1 RNA structure and function suggest that its translation may be an important regulatory step in retrotransposition. Ty1 RNA differs from typical cellular mRNAs in that it is partitioned between translation and packaging. Moreover, the 5,700 nucleotide Ty1 RNA is an unusually long RNA in yeast, and it encodes two ORFs, the second of which is expressed only when a programmed ribosomal frameshift occurs [48]. Third, the 5′ end of Ty1 RNA, including the 53-nucleotide 5′ UTR and the first 150 nucleotides of the gag ORF, is predicted to form an extended stem-loop structure that is likely to play a repressive role in translation [49,50]. Thus, ribosomal proteins and ribosome biogenesis factors that function as RHFs could participate in the regulation of Ty1 RNA translation. However, our data suggest that a significant proportion of these RHFs do not influence Ty1 cDNA levels, and therefore are not likely to directly control Ty1 RNA translation. For example, deletions of genes encoding 60 S ribosomal subunit proteins Rpl33b, Rpl34a, and Rpl37a, 40 S subunit proteins Rps11a, Rps19a, and Rps27b, ribosome biogenesis factors Rsa3 and Utp30, and the ribosome-associated chaperone, Zuo1 did not reduce Ty1 cDNA levels substantially. In addition, none of the RHFs that encode mitochondrial ribosome proteins had a significant effect on Ty1 cDNA levels. Deletion of RHFs that are required for Gag expression or translational frameshifting from gag to pol would be expected to reduce the level of Ty1 cDNA, because the ratio of Gag to Gag-Pol is critical for Ty1 protein processing, and processing, in turn, is required for cDNA synthesis [51-54]. What then are the roles of ribosome-associated factors that don’t affect early steps in retrotransposition prior to cDNA synthesis? Perhaps they act indirectly by affecting gene expression or cell growth in ways that influence the localization of VLPs or the availability of cDNA for integration. Alternatively, ribosome-associated factors could act extra-ribosomally to influence the sub-cellular localization or fate of Ty1 RNA and associated proteins, thereby interfering with nuclear import or integration of Ty1 cDNA.
The majority of RHf genes, when deleted, result in ≤2-fold change in the level of Ty1 cDNA, suggesting that they exert their effects on retrotransposition at steps subsequent to the synthesis or accumulation of Ty1 cDNA. This set of RHf genes includes several chromatin organization genes that have a potential role in the integration of Ty1 cDNA into the host genome. Ty1 integrates into nucleosomes upstream of RNA polymerase III genes, but the chromatin determinants of this integration pattern are not known. A recent genome-wide analysis of Ty1 integration sites revealed a significant correlation between Ty1 integration hotspots and nucleosomes enriched for H3K14 acetylation and histone variant H2A.Z substitution [55]. RHf genes that act after cDNA synthesis and are known to influence chromatin organization include Snf1, Gal83, and Sip4 (components of the Snf1 complex); Caf40 and Ccr4 (components of the Ccr4-NOT core complex); Hda1 and Hda3 (components of the Hda1 deacetylase complex); Ume1 and Ume6 (components of the Rpd3L histone deacetylase complex); Ino2 and Ino4 (components of the Ino2/Ino4 transcription activator); Swr1 and Vps72 (components of the SWR1 complex, which exchanges H2A.Z for H2A in chromatin-bound nucleosomes [56,57]); and Nat4, an N(alpha)-acetyltransferase involved in the N-terminal acetylation of histone H4 and H2A [58]. These chromatin modifiers could enhance integration of Ty1 cDNA by modifying the accessibility of the target DNA. Our data indicate that Nat4 is a potent co-factor for chromosomal Ty1his3AI retrotransposition even though Ty1 cDNA levels are not decreased in a nat4Δ mutant. Thus, Nat4 may modulate Ty1 retrotransposition through its effects on the chromatin structure of the target DNA. This finding may be useful in understanding the role of Nat4 in chromatin dynamics, which is poorly understood.

Deletion of 43 RHf genes resulted in ≥2-fold decrease in endogenous Ty1 cDNA levels (Table 2). A retrotransposition defect has previously been reported for eight of the 43 corresponding rhfΔ mutants, and we verified the retrotransposition defect in seven additional rhfΔ single mutants. Thus, the reduced cDNA levels in these mutants provide independent verification that these 43 RHFs affect Ty1 elements globally, rather than having specific effects on the marked Ty1his3AI element. This class includes three genes of unknown function: DGR2, HIT1, and OCA4. A forth gene, YDL124W, encodes an evolutionarily conserved NADPH-dependent alpha-ketoamide reductase, but its cellular function has not been elucidated. However, most of these RHf genes encode proteins that are involved in RNA metabolism, raising the possibility that they affect the metabolism of Ty1 RNA or its tRNA^Met primer or trafficking of Ty1 RNA between different functions in the mobility cycle. Almost one-third of the RHfs that are required for efficient cDNA accumulation are ribosome-associated. While these RHfs could act indirectly or extraribosomally, at least a few may influence the translation of Ty1 RNA. These include ribosome biogenesis factors, Bud21, Hcr1, Loc1, and Puf6, whose absence resulted in decreased Ty1 Gag:GFP fusion protein levels despite wild-type or increased levels of Ty1 RNA (Figure 4).

The RHF Bud21, also known as Utp16, is a component of the small ribosomal subunit processosome that contains U3 snoRNA. The level of the 40 S subunit is markedly decreased in a bud21Δ mutant [59]. Hcr1 encodes eIF3j, a dual function protein involved in translation.

### Table 3 Strain names and genotypes

| Strain | Genotype | Source |
|--------|----------|--------|
| BY4741 | MATa, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0 | [73] |
| BY4742 | MATa, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0 | |
| Y9230 | MATa, can1Δ, STE2pr-URA3, lys1Δ, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0 | |
| JC3807 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566 | [31] |
| JC4436 | MATa, can1Δ, STE2pr-URA3, lys1Δ, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, rtt101ΔΔLEU2 | |
| JC4501 | MATa, can1Δ, STE2pr-URA3, lys1Δ, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, YJRWTy1-2-his3AI-MET15 | |
| JC4502 | MATa, can1Δ, STE2pr-URA3, lys1Δ, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, YJRWTy1-2-his3AI-MET15, rtt101ΔΔLEU2 | |
| JC4808 | MATa, can1Δ, STE2pr-URA3, lys1Δ, ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, YJRWTy1-2-his3AI-MET15, med1ΔΔLEU2 | |
| JC5221 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566, puf6ΔΔkanMX | |
| JC5256 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566, loc1ΔΔkanMX | |
| JC5379 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566, dbp7ΔΔkanMX | |
| JC5391 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566, bud21ΔΔkanMX | |
| JC5392 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566, hcr1ΔΔkanMX | |
| JC5394 | MATa, met15Δ0, his3Δ1, leu2Δ0, ura3Δ0, Ty1his3AI-3114, Ty1(gag::GFP)-3566, mtr4ΔΔkanMX | |

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initiation as a component of translation initiation factor 3 and in processing of 20 S pre-rRNA, a precursor of the 40 S subunit. When BUD21 or HCR1 is deleted, Gag:GFP fusion protein levels are reduced to 44 and 52% of the wild-type level, respectively (Figure 4E); however, Ty1 RNA levels are increased 11-fold and 3-fold, respectively (Figure 4C). Thus, Ty1 RNA translation may be very sensitive to mutations that perturb 40 S ribosomal subunit formation because of stable secondary structure within the 5’ UTR. Another ribosome biogenesis mutant with reduced 40 S subunit formation, bud22Δ, also has a reduced level of Ty1 Gag protein; however, Ty1 RNA is not increased in bud22Δ mutants [14]. Moreover, the ratio of p45-Gag to p49-Gag is significantly decreased in a bud22Δ mutant, but we did not observe an obvious Gag processing defect in the bud22Δ or hcr1Δ mutant. Thus, the mechanism by which BUD21 and HCR1 affect Ty1 RNA translation is likely to be different from that of BUD22. The simplest interpretation of our findings is that Bud21 and Hcr1 are necessary for efficient Ty1 RNA translation via their roles in ribosome biogenesis, although other models, including indirect effects on Gag synthesis or stability are also consistent with our data.

The RHFs Puf6 and Loc1 are required for biogenesis of the 60 S ribosomal subunit. Interestingly, both also bind ASH1 mRNA and mediate its translational repression and localization to the bud tip [60]. Another RHF that is required for Ty1 cDNA accumulation, YDL124W, also binds to ASH1 RNA [61]. In contrast to ASH1 mRNA, Ty1 RNA translation may be reduced in puf6Δ and loc1Δ mutants. Moreover, Ty1 mRNA is not localized to the bud tip like ASH1 mRNA, but it is localized to microscopically distinct cytoplasmic foci known as T bodies or retrosomes [62,63]. It is possible that Puf6 and Loc1 promote translation of Ty1 RNA simply via their effects on biogenesis of the 60 S subunit. However, Loc1 and Puf6 have been implicated in the localization of specific ribosomal protein paralogs and the formation of ‘specialized’ ribosomes that are required for the regulated translation of ASH1 mRNA [64]. Based on this model, it is also conceivable that Loc1 and Puf6 are involved in the formation of ribosomes containing specific ribosomal paralogs that are necessary for the regulated translation of Ty1 RNA. A third possibility is that Loc1 and Puf6 bind Ty1 RNA directly and influence its translation or localization in the cell.

In contrast to the other ribosome biogenesis factors that we analyzed, Ty1 Gag-GFP levels were not decreased in the dbp7Δ and mrt4Δ mutants (Figure 4E), but Ty1 RNA is elevated >80-fold and >30-fold, respectively (Figure 4C). Thus, the translational efficiency of Ty1 RNA could be reduced in these mutants. Dbp7 is a putative ATP-dependent RNA helicase required for formation of mature 25 S rRNA, an RNA component of 60 S ribosomal subunits. Mrt4 is a paralog of RPP0, which encodes P0, an rRNA binding component of the ribosomal stalk. The RPP1A gene, which encodes a second ribosomal stalk protein, P1, was also identified here and in a previous study as a Ty1 co-factor (Table 1). The ribosomal stalk plays an essential role in recruiting translation factors, and P0 interacts with the ribosomal translocation factor, eEF-2 [65]. Mrt4 is bound to preribosomal particles in the nucleus and is exchanged for P0 in the cytoplasm [66-68]. Amino-acid substitutions in the essential RPP0 gene block Ty1 retrotransposition, reportedly because of effects on programmed ribosomal frameshifting [69]. Thus it is reasonable to hypothesize

Table 4 Oligonucleotide primers used in this study

| Primer     | Sequence                                      |
|------------|-----------------------------------------------|
| Ty1JR2-4   | CTTCTGTATCTCTGTTAAAGTAGAATTAAAGGGCAACCTGAGAAATATTGAACTGTGCGGTATTTCACACCG |
| Ty1JR2-2 L | GTGCACCTCTGATACAATCTGCAACAAATTTGATAAGCAATGC |
| Ty1JR2-3 L | GCTTTGCTCTTATCAATTGTTGCAAGATTTGATCTGAGAGTAGCAC |
| TYBOU72    | GTGATGACAAAAACCTCTCCG                          |
| TS523A     | GGACAGATCTCCATTCGGTG                           |
| Rtt101K3   | CTATAGCAATTAGTTAGTTTAATATAAGACGTACCACTCCTGTGAATTTCACACCG |
| Rtt101K5   | TTTTACTGCTGATAATAACTCCTGTA CGTT CAC AGG AAC AAG ATT GTA CTG AGA GTG CAC |
| PJ71       | AAAAACCAACAAACAAACTCTTTGGAGATGGGAGATTTGACTGAGAGTAGCAC |
| PJ72       | GAGTGTACGGTGTTCACTTACATTTGAGTTCAGCACTCCTGTA CGTT CAC AGG AAC AAG ATT GTA CTG AGA GTG CAC |
| PJ748      | GCTTCTGTATGGCAACCAACCAAC                           |
| PJ750      | TCTGCGAAGAATCTCCCTCGTGGGA                           |
| PJ751      | GCTAAGAGCGTATGTCCTCCTATAGCAG                          |
| PJ913      | AGAAGAATGATTCTCCGACG                                 |
| PJ914      | CCAGCTTTTGTCCC                                      |
that mrt4Δ has reduced Ty1 transposition and cDNA levels because P0 association with cytoplasmic ribosomes is partially defective in the absence of Mrt4. However, we do not observe any defects in proteolytic processing in mrt4Δ mutants, which is not consistent with a defect in Ty1 frameshifting. Thus, further investigation is needed to understand the defect in retrotransposition in dpb7Δ and mrt4Δ mutants.

**Conclusions**

Iterative synthetic genetic array analysis is a powerful tool to identify genes that are required for complex phenotypic traits influenced by multiple cellular pathways. We used this strategy to identify 275 presumptive co-factors of Ty1 retrotransposon mobility, one-quarter of which were validated by independent approaches. Ty1 co-factors participate in numerous cellular pathways and include those that affect the accumulation of Ty1 cDNA and those that act at later stages in retrotransposition. Our results highlight the extensive reliance of Ty1 on host co-factors in the mobility cycle. A significant number of Ty1 co-factors are ribosome-associated, suggesting that translational regulation plays a central role in coordinating different steps in Ty1 retrotransposition.

Many Ty1 co-factors have statistically significant human homologs, underscoring the role of conserved eucaryotic cellular pathways in Ty1 retrotransposition. Screens for human genes that are required for HIV-1 replication have uncovered over 1,000 potential co-factors; however, only a relatively small fraction of these co-factors have been validated [70]. Identification of Ty1 co-factor genes that are conserved from yeast to humans can lead to the validation and characterization of human effectors of steps in retrovirus replication that are shared among LTR-retrotransposons and retroviruses and therefore likely to be essential steps in retroelement replication.

**Methods**

**Media**

Standard yeast media were used [71], except when synthetic complete (SC) medium was supplemented with G418, in which case 0.1% monosodium glutamate was used in place of ammonium sulfate. SC medium containing monosodium glutamate is referred to as SC[msg].

**Construction of SGA query strains**

The genotype of strains used in this study, all of which are derivatives of congenic strains BY4741 and BY4742, are described in Table 3. Oligonucleotide primers used in PCR-mediated gene disruption are provided in Table 4. The yeast ORF deletion collection in strain BY4741 was obtained from Research Genetics Inc. (renamed Invitrogen MapPairs, catalog no. 95401.H2P). Strain Y9230 [31] was a gift of Dr. Charles Boone. Strain JC4436 is an rtt101Δ::LEU2 derivative of Y9230. The rtt101Δ::LEU2 allele was PCR-amplified using primers Rtt101K5 and Rtt101K3 and pRS405 DNA as a template and transformed into strain Y9230. In strains JC4501 and JC4502, the 3' UTR of YJRWTy1-1.2 was marked with his3Δ1, and MET15 was inserted between YJRWTy1-1.2 and YJR030C by one-step PCR-mediated gene disruption. PCR SOEing [72] was used to synthesize a DNA fragment containing the 3’ end of Ty1his3Δ1-Δ1 [28], the MET15 gene, and genomic DNA sequences downstream of YJRWTy1-1.2.

To accomplish this, we synthesized two PCR products, one using TYBOUT2 and Ty1JR2-2 L as primers and plasmid pGTy1his3Δ1-Δ1 as a template, the other using Ty1JR2-3 L and Ty1JR2-4 as primers and pRS401 DNA as a template. The two fragments were then annealed and amplified by PCR using primers TYBOUT2 and Ty1JR2-4. The resulting 3 kb fragment was inserted into the vector, pCR2.1-TOPO using the Invitrogen TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA). The plasmid insert was verified by restriction-site mapping and sequencing. The plasmid insert was amplified using primers TYBOUT2 and Ty1JR2-4 and the resulting DNA fragment was transformed into strains Y9230 and JC4436 by one-step gene disruption to yield strains JC4501 and JC4502, respectively. The med1Δ::LEU2 allele in strain JC4808 was constructed by PCR using primers PJ71 and PJ72 and pRS405 as template DNA. The resulting PCR product was transformed into JC4501 to yield JC4808.

Strains JC5221, JC5256, JC5379, JC5391, JC5392, and JC5394 were constructed by amplifying the appropriate orfΔ::kanMX allele from the MATa deletion collection and transforming strain JC3807 with the PCR product. All strains constructed by PCR-mediated gene disruption were checked for precise replacement of the wild-type allele by the PCR fragment using at least two diagnostic PCR reactions: one with a set of primers that flank the ORF and another with a flanking primer and a primer that hybridizes to kanMX sequences.

**Modified SGA analysis**

We used a modification of the SGA protocol of Tong and Boone [31] to accommodate a liquid medium platform and a semi-quantitative assay of Ty1his3Δ1 retrotransposition in each viable haploid strain. Trials 1 and 2 (using strain JC4502 as a query) were performed with a Thermo Scientific Matrix Hydra DT liquid handling robot. Trials 3 and 4 (using JC4808 as a query) were performed using a Beckman Coulter Biomek FX liquid handling robot.

Using a slot-pin replicator, yeast ORF deletions strains were inoculated into 96-well plates containing 200 μL YPD broth with 200 μg/mL G418 in each well. Plates were incubated at 30° for 2 days. The query strain
(JC4502 or JC4808) was grown in YEPD broth at 30\(^\circ\)C overnight. Strains were mated by transferring 5 \(\mu\)L of each ORF deletion strain and 5 \(\mu\)L of the query strain into 200 \(\mu\)L YPD broth and incubating at 30\(^\circ\)C for 3 days. To select diploids, 5 \(\mu\)L of each mating mixture was transferred to 200 \(\mu\)L SC[msg]-Met-Leu + 200 \(\mu\)g/mL G418 broth, and cultures were incubated at 30\(^\circ\)C for 3 days. Cultures of diploid strains (5 \(\mu\)L) were transferred into 200 \(\mu\)L sporulation medium + His + Ura and incubated for 14 days at 24\(^\circ\)C. Duplicate 5 \(\mu\)L aliquots of each spore culture were transferred into 200 \(\mu\)L SC[msg]-Ura-Arg + 60 \(\mu\)g/mL canavanine broth, and cultures were incubated at 30\(^\circ\)C for 5 days. Subsequently, 5 \(\mu\)L of each culture was transferred to 200 \(\mu\)L SC[msg]-Met-Leu + 60 \(\mu\)g/mL canavanine + 200 \(\mu\)g/mL G418, and cultures were incubated at 30\(^\circ\)C for 5 days. A 5 \(\mu\)L aliquot of each culture was transferred into 200 \(\mu\)L of YPD + 200 \(\mu\)g/mL G418 broth and incubated at 20\(^\circ\)C for 5 days. In one trial with strain JC4502 and one trial with strain JC4808, the appropriate parental query strain was added to an empty well in each plate at the same dilution (that is, 5 \(\mu\)L of an overnight culture in 200 \(\mu\)L YPD + 200 \(\mu\)g/mL G418 broth). Finally, 10 \(\mu\)L of each culture was spotted by hand onto YPD + 200 \(\mu\)g/ml G418 agar and onto SC-His agar (query strain JC4502), or 20 \(\mu\)L of each culture was spotted robotically (query strain JC4808), and all plates were incubated at 30\(^\circ\)C for 4 days. Duplicate plates were assigned to Trial 1 or Trail 2 (JC4502 query strain) or Trial 3 or Trial 4 (JC4808 query strain). Growth on YPD + G418 was evaluated and recorded, and retrotransposition was evaluated by individually counting His\(^+\) papillae at each address on SC-His record and retrotransposition was evaluated by individual aliquots of each spore culture were transferred into 200 \(\mu\)L SC[msg]-Ura-Arg + 60 \(\mu\)g/mL canavanine, and cultures were incubated at 30\(^\circ\)C for 5 days. Additionally, 5 \(\mu\)L of each culture was transferred to 200 \(\mu\)L SC[msg]-Met-Leu + 200 \(\mu\)g/mL G418, and cultures were incubated at 30\(^\circ\)C for 5 days. A 5 \(\mu\)L aliquot of each culture was transferred into 200 \(\mu\)L of YPD + 200 \(\mu\)g/mL G418 broth and incubated at 20\(^\circ\)C for 5 days. In one trial with strain JC4502 and one trial with strain JC4808, the appropriate parental query strain was added to an empty well in each plate at the same dilution (that is, 5 \(\mu\)L of an overnight culture in 200 \(\mu\)L YPD + 200 \(\mu\)g/mL G418 broth). Finally, 10 \(\mu\)L of each culture was spotted by hand onto YPD + 200 \(\mu\)g/ml G418 agar and onto SC-His agar (query strain JC4502), or 20 \(\mu\)L of each culture was spotted robotically (query strain JC4808), and all plates were incubated at 30\(^\circ\)C for 4 days. Duplicate plates were assigned to Trial 1 or Trail 2 (JC4502 query strain) or Trial 3 or Trial 4 (JC4808 query strain). Growth on YPD + G418 was evaluated and recorded, and retrotransposition was evaluated by individually counting His\(^+\) papillae at each address on SC-His agar. Results were tracked using an MS Excel spreadsheet and an MS Access database.

To determine the probability that RHF s identified by screening with one query strain would also be identified in the other screen with a second query strain, we calculated the hypergeometric distribution (http://www.alewand.de/statlab/tabdiske.htm). The list of 275 candidate RHF genes was submitted to FunSpec (http://funspec.med.utoronto.ca), and the statistical significance of values for enrichment in MIPS functional categories were obtained using the Bonferroni correction.

cDNA analysis

The level of unintegrated Ty1 cDNA relative to genomic Ty1 element DNA was determined by the method of Lee et al. [34], with minor alterations. Independent colonies of each strain were inoculated into 10 mL YPD broth, and each culture was incubated at 20\(^\circ\)C for 2 days. Genomic DNA prepared from each culture was digested with SphI. Ty1 cDNA was detected by Southern blot analysis using a \(^{32}\)P-labeled TYBI riboprobe. The Ty1 cDNA band was quantified relative to two genomic Ty1 bands by phosphorimaging, as described previously [28].

Northern blot analysis

Total RNA was prepared from cells grown to mid-log phase at 20\(^\circ\)C, denatured by the addition of glyoxal, separated on a 1% agarose gel and transferred to a Hybond N membrane (Amersham) as described previously [74]. Plasmids pGEM-TYA1 and pGEM-PYK1 [75] were used as DNA templates for riboprobe synthesis. Bands were quantified by phosphorimaging.

Western blot analysis

Strains were grown in YEPD broth at 20\(^\circ\)C to mid-log phase and four A600 units of cells were pelleted. Proteins were extracted from the cell pellet by the addition of 200 \(\mu\)L of lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1.8 mM MgCl\(_2\), 0.5% IGEPA L CA-630 (Sigma-Aldrich), cOmplete Mini EDTA-free protease inhibitor (Roche), 1 mM DTT, 80 U/mL RNasin (Promega)) and 200 \(\mu\)L of acid-washed beads followed by vortexing for 4X 3 min with a 3-min incubation on ice between each vortexing. A 45-\(\mu\)L aliquot of the supernatant was removed to which 5 \(\mu\)L of 5X SDS-PAGE loading buffer was added. The samples were incubated at 70\(^\circ\)C for 10 min and 6 \(\mu\)L of the supernatant was separated on a 10% SDS-PAGE gel. The proteins were transferred to a PVDF membrane and the membrane was incubated in 5% non-fat dry milk and 1X PBST for 1 h, followed by a 1-h incubation with aptitude-purified anti-Gag polyclonal antibody diluted 1:2,000 in 1% non-fat dry milk in 1XPBST or anti-Tubulin polyclonal antibody (Chemicon International) diluted 1:10,000 in 2.5% non-fat dry milk in 1X PBST as a loading control. Subsequently, the membrane was incubated with HRP-conjugated secondary antibodies and SuperSignal\textsuperscript{\textregistered} West Pico Chemiluminescent Substrate (Pierce) and exposed to film.

Retromobility frequency assays

The frequency of Ty1his3A1 retrotransposition in strains JC3807, JC5221, JC5256, JC5379, JC5391, JC5392, and JC5394 was determined by inoculating YPD broth with a single colony of each strain. The cultures were grown to saturation at 30\(^\circ\), diluted 1:1,000 in YPD broth and incubated at 20\(^\circ\)C until saturation (6 days for the dbp7\(\Delta\) derivative of JC3807; 3 days for all other strains). A 1:1,000 dilution of a 1 \(\mu\)L aliquot of each strain was plated on YPD agar to determine the titer of the culture. One milliliter aliquots of the remaining culture were plated on SC-His agar. All plates were incubated at 30\(^\circ\)C for 3 days, and the number of colonies on each plate was counted. The retromobility frequency is the number of His\(^+\) colonies divided by the total number of cells plated on SC-His agar. The average frequency and standard error
for each strain were calculated from nine to fifteen cultures.

Quantitative real-time PCR

Three independent yeast colonies of each strain were grown overnight in YPD broth at 30°C. Cultures were diluted 1:25 in YPD and incubated at 20°C for 3 h. Cells were pelleted, washed in ice-cold water, pelleted again and frozen on dry ice. Cell pellets were thawed on ice, pelleted, washed in ice-cold water, pelleted again.

Three independent yeast colonies of each strain were calculated from nine to fifteen biological replicates of each strain:

\[
\text{Fold change in Ty1 RNA} = \frac{E_{\text{Ty1}}^{\text{WT Ct}_{\text{Ty1}}}}{E_{\text{SNR6}}^{\text{WT Ct}_{\text{SNR6}}}}
\]

In the case of the wild-type strain, the fold-change was 1.0. The mean of the fold-change in the Ty1 RNA in each mutant strain relative to the wild-type strain in three sets of biological replicates of each strain was determined, and the standard error of the mean was calculated.

Additional files

Additional file 1: Table of RHF genes, human homologs and Ty1 cDNA levels in rhfΔ mutants.

Additional file 2: Tables of GO function and GO process categories of RHF genes.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

JR participated in the design of the experiment and carried out the SGA screen and constructed the databases. AK and EG carried out the assay for Ty1 cDNA levels in rhfΔ mutants. RP made strains lacking ribosome biogenesis mutants and carried out the molecular genetic assays on these mutants. MJC conceived the study, participated in the design and wrote the manuscript. All authors read and approved the manuscript.

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