

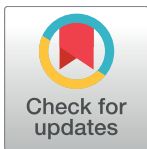
RESEARCH ARTICLE

Control of yeast retrotransposons mediated through nucleoporin evolution

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Abstract

Yeasts serve as hosts to several types of genetic parasites. Few studies have addressed the evolutionary trajectory of yeast genes that control the stable co-existence of these parasites with their host cell. In *Saccharomyces* yeasts, the retrovirus-like Ty retrotransposons must access the nucleus. We show that several genes encoding components of the yeast nuclear pore complex have experienced natural selection for substitutions that change the encoded protein sequence. By replacing these *S. cerevisiae* genes with orthologs from other *Saccharomyces* species, we discovered that natural sequence changes have affected the mobility of Ty retrotransposons. Specifically, changing the genetic sequence of *NUP84* or *NUP82* to match that of other *Saccharomyces* species alters the mobility of *S. cerevisiae* Ty1 and Ty3. Importantly, all tested housekeeping functions of *NUP84* and *NUP82* remained equivalent across species. Signatures of natural selection, resulting in altered interactions with viruses and parasitic genetic elements, are common in host defense proteins. Yet, few instances have been documented in essential housekeeping proteins. The nuclear pore complex is the gatekeeper of the nucleus. This study shows how the evolution of this large, ubiquitous eukaryotic complex can alter the replication of a molecular parasite, but concurrently maintain essential host functionalities regarding nucleocytoplasmic trafficking.

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Author summary

Genomes are the blueprint of life, but they are also plagued by parasites. Genomic parasites are elements like transposons, which are strings of genetic sequence with the capability of propagating through genomes. It is interesting to consider how organisms evolve to protect their genomes from the unchecked propagation of transposons. Here, we show that the genes encoding certain nuclear pore components in yeast have evolved to alter the mobility of Ty retrotransposons. We investigate the evolutionary and functional outcomes of this relationship, and speculate on how such a conserved structure as the nuclear pore could afford to change protein sequence while still performing its conserved functions.

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Introduction

The presence of Ty retrotransposons (Tys) in all species of *Saccharomyces* yeasts suggest that they have likely been coevolving together for about 20 million years [1,2]. Because Tys are strictly intracellular parasites, both the host (yeast) and Tys are aligned in benefitting from a controlled, sustained relationship that does not place the host at an evolutionary disadvantage [3]. This might even be thought of as a symbiotic relationship because, unlike most pathogenic viruses of higher eukaryotes, Tys are a force for genetic plasticity, driving adaptive changes within the yeast genome in response to changes in environmental conditions [4]. For this reason, it is thought that both Tys and the host genome have evolved mechanisms to attenuate unchecked Ty replication that would place an excessive burden on the host cell [3,5–10]. Thus, yeasts have likely experienced selection to control genetic parasites [11,12]. In turn, Tys may counter-adapt to evade host control strategies, or may adapt to modulate their own pathogenicity. Regardless of whether a Ty is thought of as a symbiont, or a “tamed” parasite, one can imagine that the host-parasite relationship must be finely tuned within each yeast species, with different evolutionary strategies emerging over evolutionary time (in both yeast and Ty) to control Ty replication.

There are many examples of genetic parasites, including viruses and transposable elements, that must access the nucleus of a host cell in order to replicate. Thus, the nuclear envelope represents a major barrier to these parasites in their eukaryotic hosts [13–15]. The movement of large macromolecules between the cytoplasm and the nucleus occurs through the nuclear pore complex. The nuclear pore complex is composed of multiple copies of approximately 30 different proteins, referred to as nucleoporins, and is conserved between yeast and higher eukaryotic species, including humans [16–22]. Transport receptors, called karyopherins, facilitate the transport of cellular cargo through the nuclear pore [20,23]. Genetic parasites interact with a wide variety of nucleoporins and karyopherins to facilitate the nucleocytoplasmic transport of their proteins and complexes, or to re-localize useful or antagonistic host proteins [24–33].

Saccharomyces yeasts are eukaryotes that play host to a variety of DNA plasmids, single-stranded RNA viruses (from the family *Narnaviridae*), double stranded RNA viruses (from the family *Totiviridae*), and Ty retrotransposons [34–36]. Of these viruses and virus-like elements, only Tys transit through the nuclear pore complex. There are five families of Tys in *S. cerevisiae*, Ty1 to Ty5, and all have an analogous lifecycle to retroviruses [37–39]. Tys have intracellular lifecycles (Fig 1), but can be transmitted to new hosts via yeast mating [40]. The Ty lifecycle involves the movement of Ty components between the cytoplasm and the nucleus every replication cycle via the nuclear pore complex. Ty3 virus-like particles and proteins have been observed to cluster at the nuclear envelope and the cytoplasmic face of the nuclear pore complex [25]. Multiple Ty3 proteins (Gag3, p27 and CA) interact directly with nucleoporins, and the Ty1 and Ty3 integrase (IN) proteins contain nuclear localization signals [25,41–44]. Together, these factors presumably direct the nuclear ingress of Ty cDNA and associated proteins. After nuclear entry, integrase catalyzes the insertion of Ty cDNA into the host genome [45,46]. Tys must also exit the nucleus. Ty1 RNAs, after transcription in the nucleus, are thought to be stabilized and chaperoned from the nucleus by the Gag protein [47].

Because the lifecycle of Ty involves trafficking in and out of the nucleus, we investigated the hypothesis that nucleoporins might experience evolutionary pressure to control Ty nucleocytoplasmic transport. While evolution of host immune strategies is common [48–50], evolved resistances have not been extensively documented in large, essential cellular assemblages, such as the nuclear pore complex. Seven published high-throughput screens have been conducted in order to identify genes important for the replication of Ty1 (five studies [51–55]) or Ty3 (two studies [56,57]). Among these studies, ten nucleoporins (Fig 2A) and four karyopherins

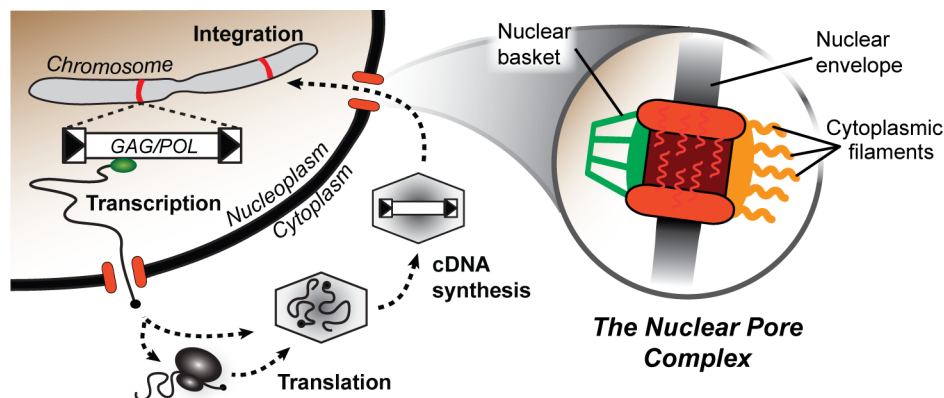


Fig 1. The nuclear pore complex is important for Ty retrotransposition. *Left.* A generic schematic of the lifecycle of a Ty. Chromosomal copies of Ty, found in the yeast genome, produce full-length RNA transcripts that are exported from the nucleus. These transcripts are translated and also packaged within virus-like particles within the cytoplasm. Packaged RNAs are reverse transcribed into cDNA that is transported into the nucleus via the nuclear pore complex. The Ty integrase mediates insertion of the cDNA into the host genome at a new location (red stripes on the chromosome). *Right.* Simplified representation of the nuclear pore complex embedded in the nuclear envelope and sliced along its vertical axis. Filaments rich in phenylalanine and glycine (FG) radiate into the nucleoplasm, cytoplasm, and within the nuclear pore itself.

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(S1 Fig) were identified as important for Ty replication. Several genes were identified in multiple screens, as represented in Figs 2A and S1. Interestingly, the knockout of some nuclear pore-related genes has been noted to reduce Ty mobility, while the knockout of others increases it [58]. One possible interpretation of this intriguing pattern is that there is a highly evolved relationship between yeasts and Tys. In some cases, Tys are successfully exploiting a nuclear pore protein for import/export. Knockout of such genes would reduce Ty mobility. In other cases the host may have evolved to reduce Ty transport, for instance by evolving a nuclear pore protein that binds but does not transit Ty componentry, or that binds Ty componentry and mis-localizes it. Deleting these genes would increase Ty mobility. There are likely to be additional nuclear pore complex-related genes, beyond those shown in Figs 2A and S1, that are involved in Ty replication. This is because genes essential to yeast viability are usually underrepresented in such screens, given that gene knockouts of these genes are inviable.

To further explore the idea of evolved control of Tys, we looked at the evolutionary history of all known *Saccharomyces* nucleoporin genes, and found that 26 of 30 nucleoporins have changed very little during *Saccharomyces* speciation and are evolving under purifying selection. However, four nucleoporins are evolving rapidly in a manner consistent with positive selection (*NUP1*, *NUP82*, *NUP84*, and *NUP116*). We wished to explore how the high level of sequence divergence in these proteins between species affects Ty control. For *NUP82* and *NUP84*, we engineered *S. cerevisiae* strains to express orthologs from other yeast species and then assayed the replicative success of different families of Tys within these otherwise isogenic yeast strains. We found that species-specific evolutionary differences in these nucleoporins affected the replication of either Ty1, Ty3, or both Ty families. *NUP84* appears to have experienced selection primarily to control Ty1, while *NUP82* has experienced selection primarily to control Ty3. Moreover, Nup82p and Nup84p are integral to the nuclear pore complex structure and are required for its functionality [59,60]. We find that adaptive changes in *NUP82* and *NUP84* affect Ty replication, yet have accumulated under the constraints of strict conservation of nucleoporin host functions during *Saccharomyces* speciation.

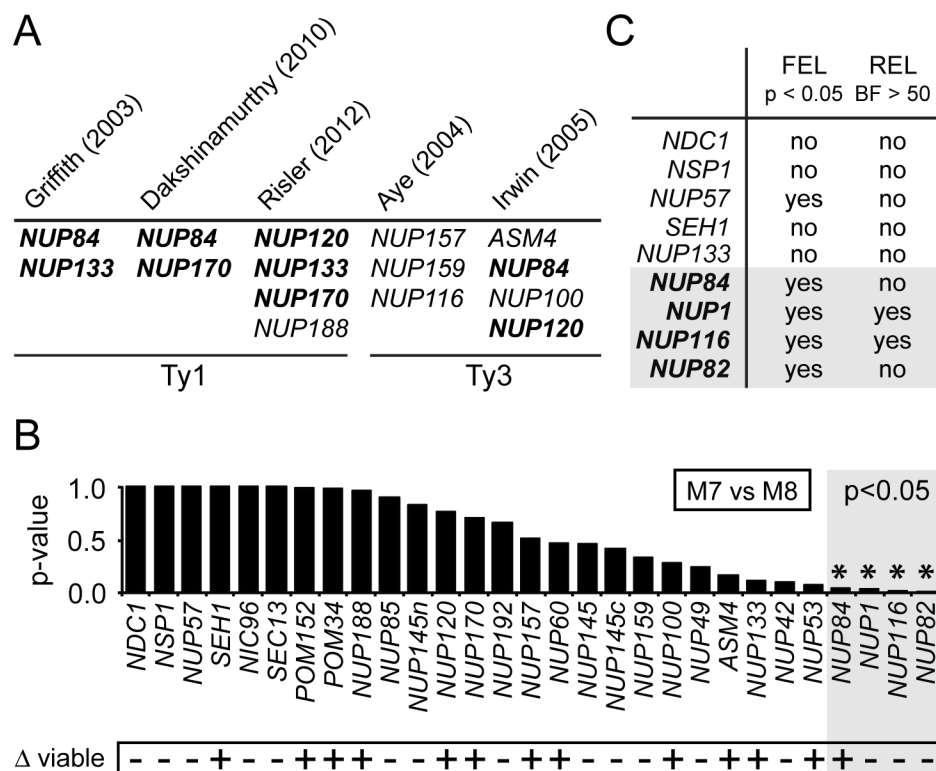


Fig 2. Nucleoporins are evolving rapidly in *Saccharomyces* yeasts. (A) Results of published high-throughput genetic screens for host factors affecting Ty mobility [51,52,55–57]. Only nucleoporin genes found in these screens are summarized, where disruption of the indicated gene altered Ty3 or Ty1 mobility. Bold text indicates genes found in more than one screen. (B) Results from PAML analysis surveying nucleoporin genes for codons with elevated evolutionary rate ($dN/dS \geq 1$). Here, alignments were fit to a codon model of conservative evolution (M7) and a codon model allowing for codons with an elevated evolutionary rate (M8). M7 was rejected in favor of M8 for four nucleoporins ($p < 0.05$): *NUP84*, *NUP1*, *NUP116* and *NUP82*. Along the bottom is summarized whether yeast with a deletion of each of these genes is viable, taken from the *Saccharomyces* genome database. (C) Extended evolutionary analysis of selected nucleoporins using two additional tests for positive selection (FEL and REL) [68]. “Yes” indicates that codons with $dN/dS > 1$ were detected in this gene by the indicated test, with a p-value (p) < 0.05, or Bayes factor (BF) > 50.

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Results

NUP82 and *NUP84* have accumulated elevated levels of non-synonymous substitutions

We first set out to determine which nuclear pore complex-related genes might be important in the evolved control of Tys. Obviously, genes that have remained unchanged over the speciation of *Saccharomyces* yeast would be unlikely to fall into this class. Instead, as a screening tool we sought genes that have diverged significantly in sequence from one yeast species to the next. We are particularly interested in genes with evidence for natural selection underlying these sequence changes, rather than genes that have diverged in sequence simply by the forces of random genetic drift. Natural selection can be detected in genes as follows. Typically, selection operates on non-synonymous substitutions (changing the encoded amino acid) more significantly than on non-synonymous mutations (silent, not changing the encoded amino acids). Gene regions that have experienced repeated rounds of natural selection in favor of protein-altering mutation therefore exhibit a characteristic inflation of the rate of non-synonymous (dN) DNA substitutions compared to synonymous (dS) substitutions (denoted by $dN/dS > 1$).

[61]. Because non-synonymous mutations occur more often than synonymous mutations by random chance, computational models have been developed that use statistical frameworks to account for these unequal substitution rates [62–64]. The mode of evolution that we are seeking ($dN/dS > 1$) is considered to be somewhat rare in eukaryotic genes. Instead, most genes experience purifying selection ($dN/dS < 1$), where protein sequence is conserved over evolutionary time due to the important and complex roles that most proteins play in cellular homeostasis.

We examined the evolution of 29 yeast genes encoding nucleoporins and 22 genes encoding karyopherins for evidence of codons with $dN/dS > 1$. For each gene, we gathered nucleotide sequences from six divergent *Saccharomyces* species (*S. cerevisiae*, *S. paradoxus*, *S. mikatae*, *S. kudriavzevii*, *S. arboricolus* and *S. bayanus*) [65–67]. Next, we constructed DNA alignments of the various genes and fit these to two different models of codon evolution using the Phylogenetic Analysis by Maximum Likelihood (PAML) package [64]. Evolutionary model M7 was used as our null model and assumes that all codons within a gene are evolving conservatively ($dN/dS > 1$ not allowed), whereas model M8 allows for some codons to exhibit an elevated evolutionary rate ($dN/dS \geq 1$). Model M7 was rejected in favor of M8 ($p < 0.05$) for four nucleoporin genes: *NUP84*, *NUP1*, *NUP116* and *NUP82* (Fig 2B). The null model was not rejected for any karyopherins (S1 Fig). Interestingly, one of these nucleoporin genes, *NUP84*, is also the only nuclear pore-related gene found in three different knockout screens as important for Ty mobility (Fig 2A). *NUP133*, *NUP120*, and *NUP170*, which were found in two independent genetic screens (Fig 2A) did not pass the threshold of significance ($p > 0.05$; Fig 2B), and so were not investigated further. The remaining three nucleoporin genes under positive selection (*NUP1*, *NUP116* and *NUP82*) are essential genes within *S. cerevisiae* (Fig 2B, bottom), and of these, only *NUP116* has been directly tested and demonstrated to be involved with Ty replication [25].

Various statistical tests have been designed to detect positive selection, all of which take different approaches to modeling the rates of nonsynonymous and synonymous changes that have occurred in a given gene alignment [69]. We next evaluated *NUP84*, *NUP1*, *NUP116*, and *NUP82* with additional tests for positive selection, FEL and REL [68]. We found that all four nucleoporin genes showed evidence of positive selection using at least one of these additional tests (Fig 2C). Furthermore, three of these genes (*NUP1*, *NUP82*, and *NUP116*) were previously identified as evolving rapidly in a whole genome evolutionary study of five *Saccharomyces* yeast species performed by Scannell *et al.* [67]. In contrast, *NUP133* and four other nucleoporins with the least support for rejection of the M7 null model (*NDC1*, *NSP1*, *NUP57* and *SEH1*; Fig 2B), passed zero or only one of these tests (Fig 2C). We next turned to functionally testing the biological relevance of the observed evolutionary signatures identified within nuclear pore complex-related genes.

A novel GFP reporter of Ty mobility

We first built a quantitative, GFP-based assay system for Ty mobility, which is a variation of a previous assay used in this field [70]. In this system, a plasmid-mounted Ty1 genome from *Saccharomyces cerevisiae* was encoded on the Watson (sense) strand, and was engineered to contain an internal *GFP* gene on the Crick (anti-sense relative to the transcript) strand of the DNA (Fig 3A). To prevent its expression directly from the plasmid vector, the *GFP* gene was engineered to contain an antisense intron (on the Watson strand). Thus, only after the full-length Ty1-GFP transcript has been spliced, reverse transcribed, and integrated into the *S. cerevisiae* genome can the *GFP* gene be expressed. *GFP* expression is regulated by the inducible copper-sensitive *CUP1* promoter (Fig 3A). Experiments were performed with two different

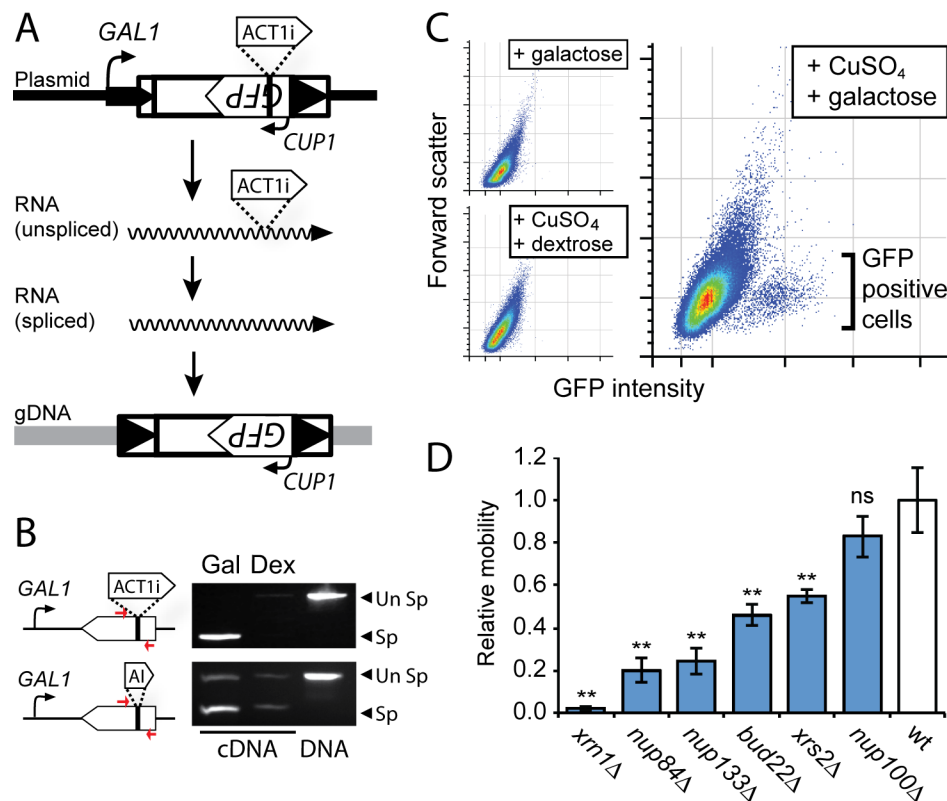


Fig 3. A novel GFP-based reporter of Ty1 mobility. (A) An overview of the GFP-tagged Ty1 plasmid. Ty1 transcription is induced by activation of the *GAL1* promoter that produces a long Ty1 transcript including an internal *GFP* gene and an *ACT1* intron (*ACT1i*). The spliced transcript has the *ACT1i* removed, which then provides a template for Ty1 protein production and reverse transcription. Ty1 cDNA is imported into the nucleus and integrated into the *S. cerevisiae* genome. The *GFP* gene is then induced from the *CUP1* promoter by *CuSO₄* to report successful integration events. (B) RT-PCR was used to assess splicing of RNA with *ACT1i* versus an artificial intron (AI) within the *GFP* gene (primer positions marked by red arrows). Spliced RNA transcripts (Sp) were mainly detected upon induction of the transcription by the *GAL1* promoter using galactose (Gal). Growth on dextrose (Dex) inhibits the *GAL1* promoter and the production of RNA transcripts. Plasmid DNA was used as a positive control to allow the PCR amplification across intron-containing *GFP*. "Un Sp" indicated the detection of unspliced RNAs. (C) Flow cytometry analysis shows that *GFP* is only expressed under conditions of galactose induction of Ty1 expression followed by *CuSO₄* induction of *GFP*. (D) The effect of six different gene deletions on Ty1 mobility, relative to wild-type *S. cerevisiae*. The relative mobility was measured as a percent of GFP positive cells after induction of the Ty1-GFP reporter, and was repeated independently, three times (error bars: standard error, $n > 3$; **Tukey-Kramer method, $p < 0.05$). All values are normalized to wildtype.

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introns within the *GFP* gene in order to determine which was more efficiently spliced from the transcript produced. The more efficient splicing occurred using the *S. cerevisiae* *ACT1* intron (*ACT1i*) (Fig 3B). GFP-positive cells were only detected by flow cytometry after galactose was added to the growth medium to initiate Ty1 transcription, and subsequent addition of *CuSO₄* to induce expression of the *GFP* reporter (Fig 3C). We tested our Ty1 mobility reporter in isogenic strains deleted for five genes known to be important for efficient Ty1 mobility: BY4741 *xrn1Δ*, *nup84Δ*, *nup133Δ*, *bud22Δ*, and *xrs2Δ* [51,52,55]. Indeed, we see a significant decrease in Ty1 mobility in each deletion strain compared to the wild-type BY4741 background (Fig 3D). As a control, we show that a strain deleted for *NUP100*, which is important for Ty3 mobility [56], but not known to be important for Ty1, supports a level of mobility that is not significantly different from that of a wild-type strain (Fig 3D).

NUP84 evolution modulates Ty1 mobility within *S. cerevisiae*

NUP84 is under positive selection and disruption of the gene affects both Ty1 and Ty3 replication (Fig 2). We wished to test whether the evolution of *NUP84* over yeast speciation has altered interactions with Tys. To test this, we replaced *NUP84* within the *S. cerevisiae* genome (*NUP84^{S.cer}*) with *NUP84* from diverse *Saccharomyces* species (*S. mikatae*, *S. kudriavzevii* and *S. bayanus*) as outlined in Fig 4A. These sequences encode Nup84p that are between 88% (*S. mikatae*) and 85% (*S. bayanus*) identical to the *S. cerevisiae* protein. As an isogenic control, we re-complemented the *nup84Δ* strain with *S. cerevisiae* *NUP84*. Chromosomal complementation of *S. cerevisiae* *nup84Δ* with each heterospecific (other species) *NUP84* allele resulted in the restoration of normal growth and cellular morphology (Fig 4B and 4D), normal nuclear import (Fig 4C and 4D), and normal gene expression from the promoters used in our Ty1 GFP-based reporter (Fig 4E).

The null strain, and each of the four strains expressing wildtype or heterospecific *NUP84*, were transformed with the Ty1 GFP reporter described above. Relative to *nup84Δ*, cells complemented with *NUP84^{S.cer}* increased Ty1 mobility approximately 5-fold (Fig 5A). There were highly significant differences in the levels of Ty mobility among strains encoding heterospecific *NUP84* (one-way ANOVA, $p = 8.2 \times 10^{-8}$), and levels of Ty1 mobility were significantly different in strains containing *NUP84^{S.mik}*, *NUP84^{S.kud}*, and *NUP84^{S.bay}* when compared to *NUP84^{S.cer}* (Tukey–Kramer method, $p < 0.05$) (Fig 5A). We found that replacement of *NUP84^{S.cer}* with *NUP84^{S.kud}* increased Ty1 mobility by 32%, whereas *NUP84^{S.mik}* and *NUP84^{S.bay}* both significantly decreased mobility by 21% and 35%, respectively. To verify the observed differences in control of Ty1 mobility, we used Southern blotting to detect Ty1 integrations in the 5' UTR of the *SUF16* locus, as previously described [73]. We used our GFP reporter assay to initiate Ty1 mobility, with Ty1 genomic integrations only detected after induction by galactose (Fig 5B). Similar to our GFP reporter assay, fewer integrations were detected within strains encoding *NUP84^{S.mik}* and *NUP84^{S.bay}* compared to *NUP84^{S.cer}*. *NUP84^{S.cer}* and *NUP84^{S.kud}* had comparable levels of genomic integrations (Fig 5B). To further ensure the generality of our findings, we also measured Ty1 mobility on a single-copy plasmid under both high and low expression conditions. We used the integration of a *HIS3* gene as a marker of successful Ty1 mobilization by assaying the appearance of colonies able to grow on a histidine deficient medium [70]. Again, we were able to observe that heterospecific substitutions were able to alter Ty mobility, even with a 6–9 fold decrease in overall mobility from a single copy plasmid (S2 Fig). Results were broadly consistent between the three assays, with the exception of the heterospecific swap of *S. kudriavzevii* *NUP84*, which reduced Ty1 mobility in the low copy assay (S2 Fig).

These data show that evolutionary differences between *NUP84* of different *Saccharomyces* species modulate the efficiency of Ty1 mobility in a species-specific manner, even though all host functions are conserved. Pairing Ty1 from *S. cerevisiae* with *NUP84* of other species apparently decouples a finely co-evolved relationship, altering levels of Ty1 mobility. To support this model, we also assayed the impact of *NUP84* evolution on Ty3 replication. We used a galactose inducible Ty3 with a *HIS3* reporter gene and assayed the appearance of colonies able to grow on a histidine deficient medium [70,74–76]. In contrast to Ty1, we found that *nup84Δ* resulted in increased Ty3 mobility, as was previously reported [56]. However, each of the heterospecific *NUP84* genes returned transposition to the lower level with no significant difference in mobility among strains encoding heterospecific *NUP84* (one way ANOVA, $p = 0.90$) (Fig 5A). Collectively, these data suggest that the co-evolutionary dynamics are specific to *NUP84* and Ty1.

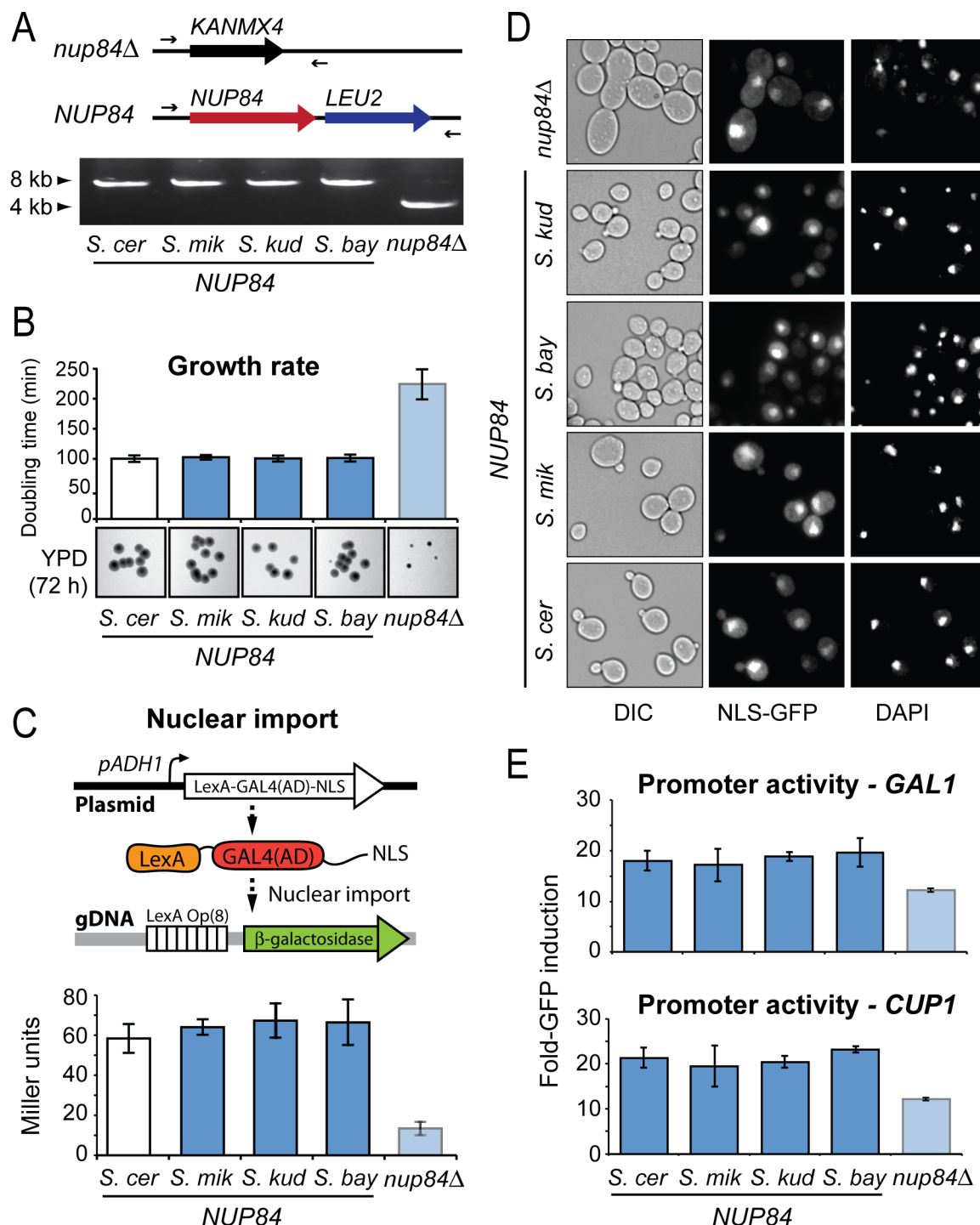


Fig 4. The housekeeping functions of *NUP84* are conserved across divergent *Saccharomyces* species. (A) Top. A schematic representation of the *NUP84* locus within *S. cerevisiae* engineered to either lack *NUP84* (*nup84Δ*) or to express heterospecific *NUP84* from *S. cerevisiae* (*S. cer*), *S. mikatae* (*S. mik*), *S. kudriavzevii* (*S. kud*) or *S. bayanus* (*S. bay*) along with the *LEU2* selectable marker. Bottom. Successful genome engineering was confirmed by PCR amplification across the *NUP84* locus to detect the replacement of *KANMX4* with *NUP84-LEU2* (primers marked as arrows). (B) The doubling time of *NUP84*-complemented strains in a liquid YPD medium compared to *nup84Δ*, and colony growth and morphology after 72 h of growth on a solid YPD medium. (C) General nuclear import function was assessed in the presence of heterospecific Nup84p or absence of Nup84p using a LexA-Gal4(AD) reporter protein with a SV40 nuclear localization signal (NLS) [71]. The LexA DNA binding domain and Gal4 activation domain (AD) initiate transcription of the β -galactosidase gene upon successful nuclear import. (D) Nuclear transport was also assessed by the steady-state

localization of a GFP reporter protein containing a NLS from *PHO4* [72] and its cellular accumulation relative to a DAPI-stained nucleus within *NUP84* complemented *S. cerevisiae*. (E) The effect of *NUP84* complementation or deletion on the ability of *S. cerevisiae* to express *GFP* from each of the promoters used in the Ty1 *GFP*-based reporter (*GAL1* (top) or *CUP1* (bottom) promoters), using mean fluorescent intensity (MFI) detected by flow cytometry (error bars: standard error, $n > 3$).

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NUP82 evolution alters both Ty1 and Ty3 mobility

Our evolutionary analysis also identified the gene *NUP82* as being the highest scoring nucleoporin in our evolutionary screen (Figs 2B and S1), however no role for *NUP82* has been reported in Ty biology. This could be because *NUP82* is an essential gene and would have eluded detection in genome-wide knockout screens. To investigate whether *NUP82* is involved in Ty replication, a dominant negative approach was adopted. Full- or partial-length portions of *NUP82* were expressed in cells that are otherwise wild type at the *NUP82* locus. These Nup82p constructs included the mutations D204A, F290A, Y295A, L393A, I397A, L402A, L405A and F410A (Nup82p^{DFY-LILLF}) that inactivate interaction with other nucleoporins and decouple it from the nuclear pore complex [77] (Fig 6A). Nup82p^{DFY-LILLF} is non-functional as a nucleoporin, therefore we reasoned that it would compete with wild-type Nup82p and have an inhibitory effect on mobility if Ty interacts with Nup82p to transit the nuclear pore. Indeed, the expression of the C-terminal helical domain of Nup82p (residues 433–713) significantly reduced Ty1 mobility, with the N-terminal β -propeller domain (residues 1–458) being dispensable for this effect (Fig 6B). Expression of any of the dominant negative *NUP82* genes did not noticeably affect the growth of *S. cerevisiae* (Fig 6C) or general nuclear import (Fig 6D) compared to expression of the control gene *MET17*, which suggests that these proteins are not toxic to *S. cerevisiae* and do not disrupt the nuclear pore complex. In summary, this serves as preliminary evidence of a previously uncharacterized role for *NUP82* in Ty1 replication.

Next, in a similar approach to that taken with *NUP84*, *S. cerevisiae* was engineered to express *NUP82* from different *Saccharomyces* species to ascertain the impact of *NUP82* evolution on Ty mobility. Due to the essential nature of *NUP82*, we used a *NUP82/nup82Δ* heterozygous diploid strain from the “synthetic genetic array” collection [78] as our starting strain for

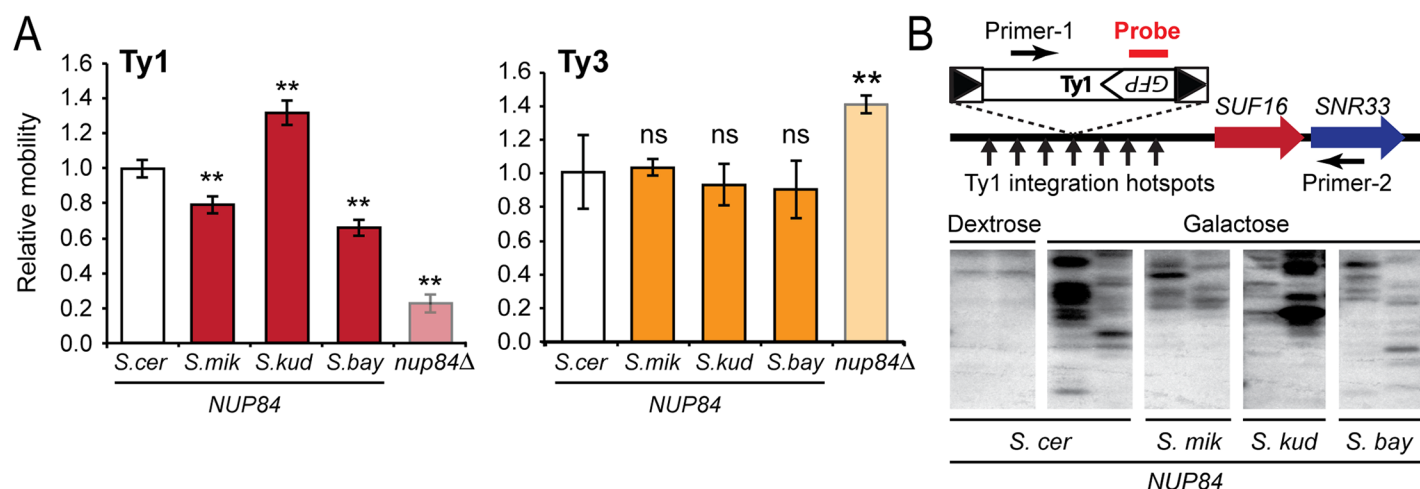


Fig 5. Evolutionary differences between *NUP84* of different *Saccharomyces* species alter levels of Ty1 mobility. (A) Relative mobility of Ty1 and Ty3 within *nup84Δ* or *nup84Δ* complemented with heterospecific *NUP84* from different *Saccharomyces* species. Asterisks designate complemented strains that have significantly different levels of mobility compared to the strain encoding *NUP84* from *S. cerevisiae* (Tukey–Kramer method, $p < 0.05$) (error bars: standard error, $n > 3$). (B) Southern blot analysis of Ty1 integration in two independent clones upstream of the *SUF16* locus, which contains Ty1 integration hotspots in its promoter [73]. PCR products across the *SUF16* locus were run on a gel and then probed with a radiolabeled DNA probe specific to *GFP* in order to detect integration events.

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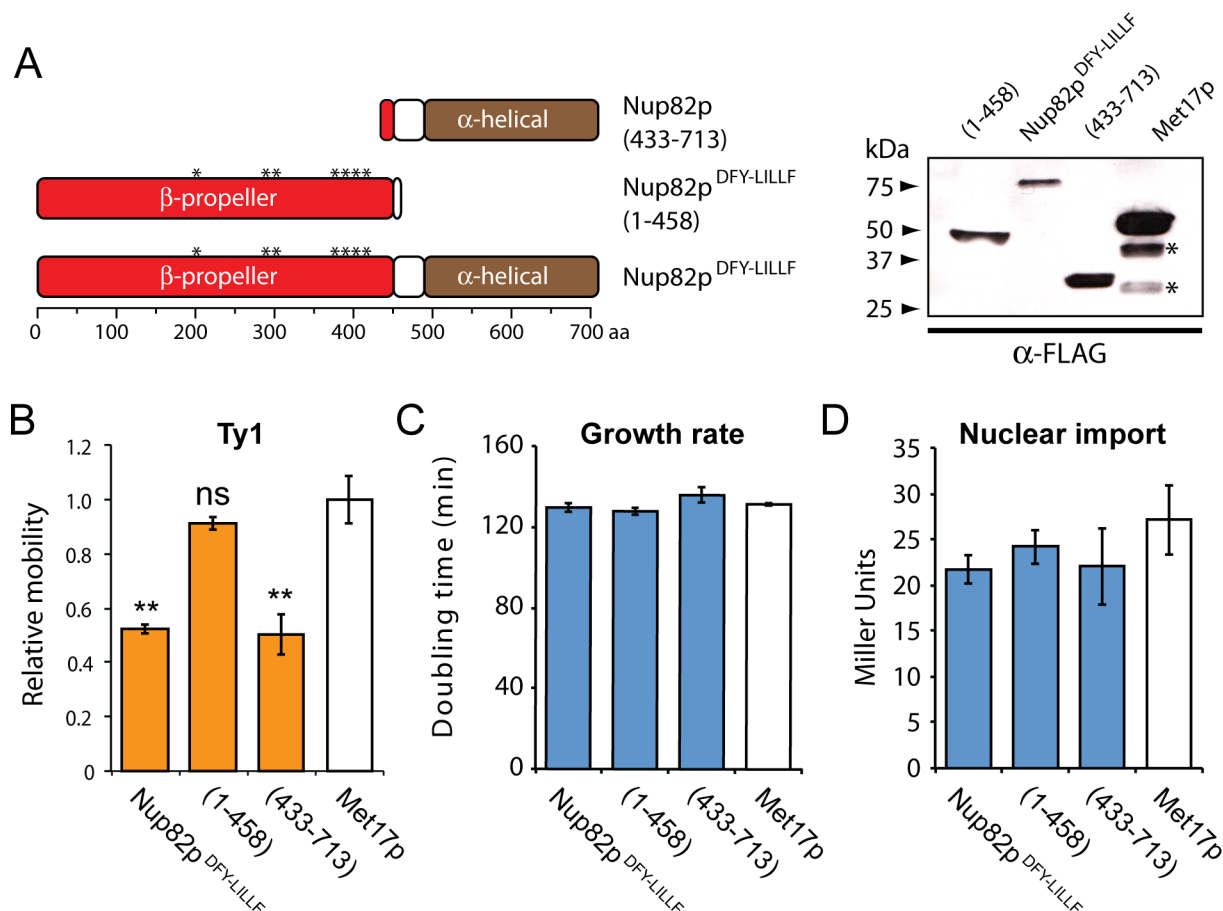


Fig 6. The expression of dominant negative NUP82 and its impact on Ty1 mobility. (A) *Left.* A linear domain diagram of Nup82p^{DFY-LILLF} and derived deletion mutants [Nup82p(433–713) and Nup82p^{DFY-LILLF}(1–458)]. Asterisks mark the mutations that decouple Nup82p from the nuclear pore complex. *Right.* Western blot analysis to detect the expression of FLAG-tagged Nup82p^{DFY-LILLF} and its derivatives, compared to the expression of a control protein (Met17p) in the wild-type background (*Met17p degradation products). The effect of Nup82p^{DFY-LILLF} expression on (B) Ty1 mobility, (C) doubling time in a liquid medium and (D) the nuclear import of the reporter protein LexA-MBP-Gal4 (AD), relative to the expression of MET17 (error bars: standard error, n>3; **Tukey–Kramer method, p<0.05).

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the genomic replacement of NUP82^{S.cer}. A customized SclI restriction endonuclease method was used to improve the efficiency of homologous recombination-based gene replacement (see [methods](#)) (Figs 7A and S3). *S. cerevisiae* encoding heterospecific NUP82 have a normal colony morphology, growth rate (Fig 7B), and no difference in GAL1 and CUP1 promoter expression (S4 Fig), suggesting that the cells are the same in measurable host functions. We tested the effect of NUP82 evolution on Ty1 mobility using the GFP fluorescence assay and, in contrast to our studies of NUP84, found that Ty1 mobility levels were similar in strains expressing NUP82^{S.mik} and NUP82^{S.bay} and NUP82^{S.cer}, but were significantly higher for strains complemented with NUP82^{S.kud} (Fig 7C). Thus, although NUP82 may be important for Ty1 mobility (Fig 6), we find that Ty1 seems mostly insensitive to the evolutionary differences between NUP82 of different species. We next assayed the replication of a Ty3 retrotransposon in the engineered NUP82 heterospecific strains. *S. cerevisiae* expressing NUP82^{S.mik} resulted in a significant >3-fold increase in Ty3 mobility, relative to NUP82^{S.cer} (Tukey–Kramer method, p<0.05) (Fig 7C). These data show that the evolutionary differences within *Saccharomyces* NUP82 can impact both Ty1 and Ty3 mobility, but predominantly Ty3. Together, we show

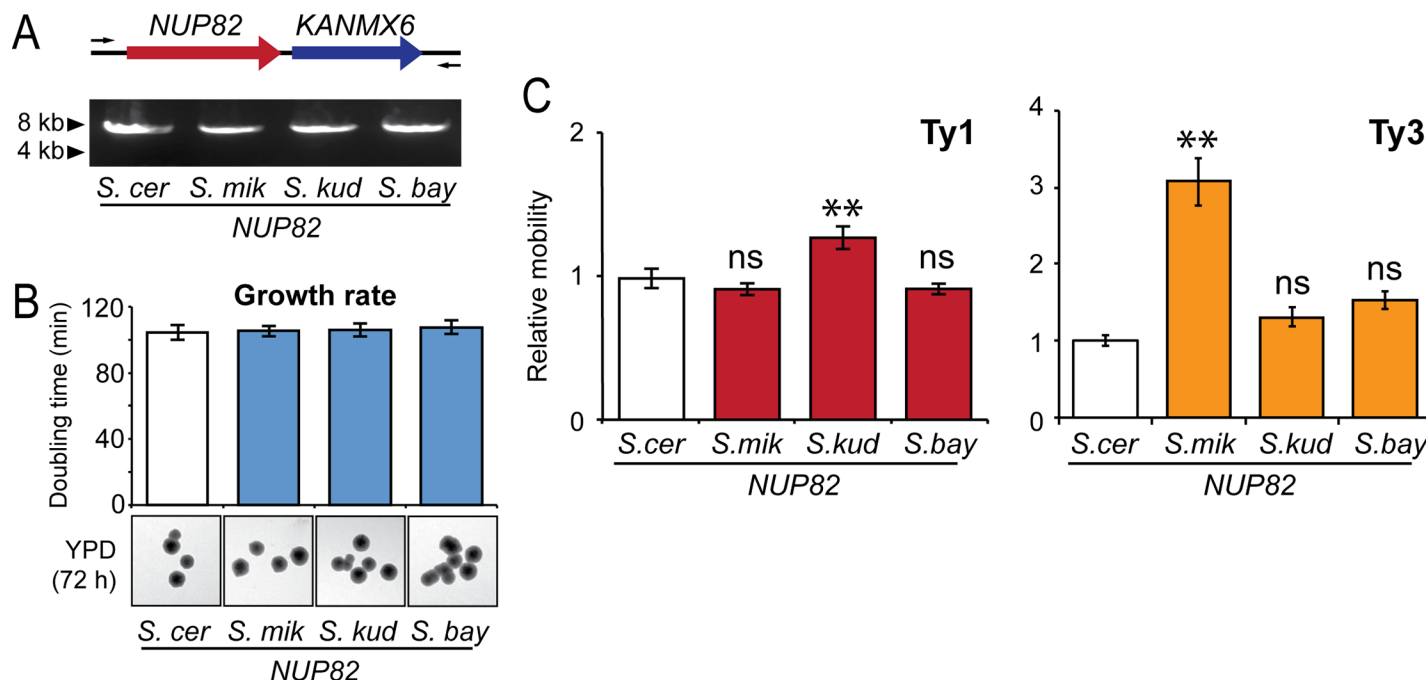


Fig 7. The evolution of *NUP82* and its effect on Ty1 and Ty3 mobility within *S. cerevisiae*. (A) A schematic representation of *S. cerevisiae* engineered to express *NUP82* from different *Saccharomyces* species. Genome engineering was confirmed by PCR amplification across the *NUP82* locus. (B) The doubling time of *NUP82*-complemented strains in a liquid medium. Colony growth and morphology of the engineered strains was monitored for 72 h on a solid YPD medium (error bars: standard error, $n > 3$). (C) Relative mobility of Ty1 and Ty3 within strains complemented with *NUP82* from different *Saccharomyces* species. Asterisks mark significant differences in Ty mobility compared to the strain encoding *NUP82* from *S. cerevisiae* (error bars: standard error, $n > 3$; **Tukey–Kramer method, $p < 0.05$).

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that *NUP82* appears to play a previously uncharacterized role in Ty mobility, and that Ty1 and Ty3 are differentially susceptible to evolutionary changes within *NUP82*.

Discussion

There are many selective pressures driving the evolution of *Saccharomyces* yeasts, including resource competition, sexual selection, and pressure to co-exist with viruses and other genetic parasites [11,12,79–83]. We find signatures of natural selection acting on several nucleoporins, coinciding with previous observations that deletion or disruption of several of these genes can alter Ty mobility. Here, we use a unique approach to demonstrate that the evolutionary changes that have naturally accumulated in yeast nucleoporins can also alter Ty mobility levels, just like laboratory perturbations of these genes are known to do. We successfully replaced *NUP82* and *NUP84*, within the context of the *S. cerevisiae* genome, with orthologs from related *Saccharomyces* yeasts, and then demonstrated altered Ty mobility in these isogenic yeast strains. While it is never possible to know for sure what has driven selection within these genes, nucleoporins from different *Saccharomyces* species support variable levels of Ty1 or Ty3 mobility, providing a phenotypic trait on which selection may have been acting. This is similar to our recent observations that the antiviral *XRN1* gene from *Saccharomyces* yeasts has likely co-evolved with totiviruses to control excessive viral replication [11].

It is important to note that, while we have explored nucleoporin evolution, the genetic parasites used in this study have been held constant, with both Ty1 and Ty3 deriving from the *S. cerevisiae* lineage. In some cases, orthologs of *NUP82* and *NUP84* resulted in higher levels of *S. cerevisiae* Ty mobility, and in other cases, they resulted in lower levels. These patterns are consistent with a model where nucleoporins and Tys are co-evolved in each species. When a

Nup82p or Nup84p ortholog is substituted within the *S. cerevisiae* nuclear pore, sometimes *S. cerevisiae* Tys can exploit it better than it can the *S. cerevisiae* version of that protein (possibly by having an increased affinity for the foreign ortholog, which is not evolved to evade *S. cerevisiae* Tys). Other times, the *S. cerevisiae* Ty is less compatible with this orthologous protein. Ultimately, our data show that the replacement of *S. cerevisiae* nucleoporins with heterospecific nucleoporins “decouples” this interaction and leads to either an increase or decrease in Ty mobility, without impacting cellular homeostasis (e.g. nuclear import). It is tempting to try to extrapolate from this study predictions that certain yeast species are better at controlling Ty retrotransposons than others, but this study alone cannot support such conclusions. In this study, we assayed only *S. cerevisiae* Tys, but if we assayed Tys from other species, we might expect them to have evolved an optimal interaction with their cognate nuclear pore machinery. Thus is the nature of evolutionary arms race dynamics [48–50].

The exact functions of *NUP82* and *NUP84* during Ty replication remain unclear. Ty nuclear ingress likely involves docking of the virus-like particle to the nuclear periphery by interaction with nucleoporins [25]. The known positioning of Nup82p and Nup84p at the cytoplasmic face of the nuclear pore complex could possibly facilitate virus-like particle docking, in a similar manner to their recruitment and binding of host karyopherins prior to nuclear import [84–87]. Multiple Ty3 proteins (Gag3, p27 and CA) interact directly with nucleoporins, and the integrase of Ty1 and Ty3 contain nuclear localization signals [25,41–44]. Therefore, it seems likely that Ty proteins interact directly with nucleoporins, making it plausible that evolutionary selection could be acting to alter these physical interactions.

The evolutionary relationship between yeast and Tys is complex. The intracellular lifecycle and ubiquity of Tys would suggest that Tys have been co-evolving with the *Saccharomyces* genus for many millions of years [1,2]. Ty copy number varies greatly between different strains and species of *Saccharomyces* yeasts and there is likely a dynamic cycle of Ty gain and loss. Indeed, certain families of Ty are completely absent from certain strains and species of *Saccharomyces* yeasts [1,65,88–90]. One example relevant to our findings is the apparent lack of Ty1 from *S. bayanus* [1], which is a complex hybrid species with genetic contributions from *S. cerevisiae*, *S. eubayanus*, and *S. uvarum* [91]. Our results show that *NUP84* from *S. bayanus* inhibits Ty1 mobility, this might have been protective against colonization by *S. cerevisiae* Ty1 during hybridization. The general persistence of Ty in *Saccharomyces* yeasts suggests that complete loss of Tys from a species is relatively rare, perhaps due to continued Ty introgression or transmission by sexual reproduction, which are potential mechanisms by which Tys can invade Ty-free or naive populations [1,90]. The error prone nature of the Ty reverse transcriptase and reverse transcription-mediated recombination can also generate Ty variants that could overcome host-encoded resistance mechanisms [92]. In contrast to the idea that Tys are completely parasitic, Ty mobility can drive the evolution of the yeast genome by changing gene regulation and expression by integrating in or near host genes. Tys can also facilitate gross chromosomal rearrangements of the host genome, including translocations and deletions, by way of homologous recombination between Ty integrated at different locations within host chromosomes [93–96]. Experimental systems have shown that Ty-mediated genome evolution can be observed in the laboratory [4], and would likely allow populations of *Saccharomyces* yeasts to rapidly respond to selective pressures found within the natural environment. Thus, in the context of the nuclear pore complex, there may be evolutionary selection to prevent Ty nuclear transit and excessive replication, but also selection against mutations that completely abrogate Ty mobility. The long-term association between *S. cerevisiae* and its cognate Tys would imply that this interaction has been optimized by evolutionary selection, perhaps to balance the damaging effects of excessive Ty mobility with the benefits of genome plasticity.

The nuclear pore is the gatekeeper of the nucleus, and it is antagonized by many pathogens and genetic parasites throughout eukaryotes. Recently, we have demonstrated that naturally occurring evolutionary differences between primate species in a nuclear pore component called RanBP2/Nup358 alter infection by simian immunodeficiency viruses (SIVs) [120]. We showed that differential interaction with RanBP2 in each host species drove SIV evolution as it transmitted between ape species, ultimately setting the stage for the zoonoses that yielded HIV-1. Remarkably, the very same nucleoporins that are under positive selection in yeast have also been shown to be essential to the replication of other retrotransposons and viruses. The fission yeast *Schizosaccharomyces pombe* ortholog of *NUP1* (*NUP124*) is required for mobility of the Ty3/gypsy-like element Tf1 and directly interacts with the Tf1-encoded Gag protein [97,98]. The human homologs of *NUP1* and *NUP116* (*NUP153* and *NUP98*, respectively) are important for viral replication in humans, including for HIV, HBV, HCV, and influenza virus [24,29,33,99–104]. Specifically, *NUP153* is an important determinant of HIV and HBV nuclear import, and its FG (phenylalanine-glycine)-repeat domain directly interacts with HIV capsid, via specific FG-repeats [24,33,105,106]. In *S. cerevisiae*, the FG-repeat region of Nup116p directly interacts *in vitro* with the Ty3-encoded protein Gag3, and truncation of *NUP116* decreases Ty3 mobility [25]. Collectively, this paints a picture of complex evolutionary pressures on nuclear pore genes across eukaryotes.

It appears that viral infections have broadly shaped the evolution of host genomes, affecting genes well beyond immunity loci [107]. The most classic example involves cellular entry receptors used by viruses to enter cells. These receptors are often under positive selection, resulting in highly species-specific interactions with viruses [108–112]. The nuclear pore complex is the gatekeeper of the nucleus just like cell surface receptors are gatekeepers of the cytoplasm. Our work in *Saccharomyces* yeasts provides a framework to further investigate the importance of the nuclear pore complex in modulating Ty mobility, and for a parallel investigation into the evolution of the orthologous nuclear pore complex of higher eukaryotes. It remains unknown how broadly viruses and genetic parasites are driving the evolution of important housekeeping proteins, but intriguing recent reports involving genes such as *XRN1* (involved in degradation of uncapped mRNAs; [11]), and DNA repair genes [113], suggest that this might be more common than previously appreciated.

Materials and methods

Plasmid construction

The *ACT1* intron (*ACT1i*) and an artificial intron (AI) [114] were amplified by PCR with included primer-encoded flanking homology to *GFP*. This PCR product was inserted directly after the ATG start codon at the 5' end of *GFP* by the “yeast plasmid construction by homologous recombination” method (recombineering) [115]. *GFP*(AI) and *GFP*(*ACT1i*) were amplified by PCR and introduced into pAG423-GAL-*ccdB* using TOPO-TA and Gateway cloning strategies (Thermo Fisher) to create pPAR061 and pPAR063, respectively. *GFP*(*ACT1i*) was also placed under the control of the *CUP1* inducible promoter (456 bp upstream of *CUP1* were cloned directly from the genome of *S. cerevisiae*) using recombineering. pCUP1-*GFP*(*ACT1i*) was used to replace *HIS3*(AI) within pGTY1-*HIS3*(AI) to create pPAR078. pPAR101, pPAR104, pPAR145 and pPAR181 were constructed by using PCR to create DNA encoding FLAG-tagged Nup82p^{DFY-LILLF} (1–458), Nup82p^{DFY-LILLF} and Nup82p (433–713) from pNOP-GFP-Nup82p^{DFY-LILLF} [77]. *MET17* was amplified directly from the genome of *S. cerevisiae*. All PCR fragments were subsequently cloned into pAG414-GPD-*ccdB* via PCR8 using TOPO-TA and Gateway cloning strategies (Thermo Fisher). To assay nuclear import using the strategy outlined by Marshall *et al.* we first subcloned the LexA-MBP-GAL4(AD) cassette from

pJMB1076n [71] into the pAG413 plasmid backbone using recombineering, essentially changing the selective marker on the plasmid from *LEU2* to *HIS3*. For gene knockout, all plasmids were constructed by recombineering using *NUP82* and *NUP84* amplified from various *Saccharomyces* species. These nucleoporin genes were placed upstream of a selectable marker (*LEU2* or *KANMX6*) and the entire cassette flanked by 1000 bp of sequence encompassing the 5' and 3' untranslated regions of *NUP82* or *NUP84* from *S. cerevisiae*. pPAR240 was constructed by first amplifying a LexA operator sequence upstream of the β -galactosidase gene from *S. cerevisiae* L40. PCR products were designed to contain flanking homology to *ADE2* from *S. cerevisiae* and these PCR products were used to disrupt the *ADE2* gene within pRS422 to create pPAR240. The DNA sequences from all constructed plasmids can be found in S2 File. A list of all relevant plasmids can be found in S1 Table. A list of all relevant yeast strains can be found in S2 Table.

Evolutionary analyses

Gene sequences from six species of *Saccharomyces* yeasts were obtained from publically available online resources. Maximum likelihood analysis of dN/dS was performed using the codeml program in PAML 4.1. Multiple protein sequence alignments were created and were manually curated to remove ambiguities before processing with PAL2NAL to produce accurate DNA alignments [116]. DNA alignments were fit to two models: M7 (codons fit to a beta distribution of dN/dS values, with dN/dS > 1 disallowed) and M8 (similar to model 7, but with dN/dS > 1 allowed). One model of codon frequency (f61) and a seed value of 0.4 for dN/dS (ω) was used (S1 File). Likelihood ratio tests were performed to evaluate which model of evolution the data fit significantly better with positive selection and inferred if we can reject M7 in favor of M8 with a $p < 0.05$. REL and FEL codon based models were also used to detect sites under positive selection as implemented by the HyPhy package using the best substitution models chosen by Akaike information criterion (AIC) using the phylogenetic tree (Newick format): (((*S. paradoxus*, *S. cerevisiae*), *S. mikatae*), *S. kudriavzevii*), *S. arboricolus*, *S. bayanus*) (S1 File).

Strain construction

Standard methodologies for PCR-based gene knockout and replacement were used to create all *NUP84* strains in BY4741 (YPAR0130-0133) [117]. Strains YPAR0135-0138 were engineered to encode a LexA operator sequence upstream of the β -galactosidase gene, and were constructed by the disruption of the genomic copy of *ADE2* using a PCR cassette amplified from pPAR240. Clones selected for their ability to grow on a medium lacking uracil and inability to grow on a medium lacking adenine. *NUP82* gene replacement utilized a *SceI*-based method to increase the efficiency of the integration of *NUP82* and *KANMX6* by generating DNA double-stranded breaks at the *NUP82* locus in *S. cerevisiae* (personal communication, Dr. C.M. Yellman). Using a diploid heterozygous knockout strain of *NUP82* [78], *KANMX6* at the *NUP82* locus was replaced with the *URA3* gene from *K. lactis* flanked by *SceI* sites amplified by PCR from pCMY-IT3. Gene replacement was carried out by the concomitant expression of *SceI* from pGAL1-SCEH and the LiAc transformation of a PCR-derived cassette encoding *NUP82-KANMX6*. *NUP82/nup82 Δ ::NUP82-KANMX6* clones were selected by their ability to grow in the presence of 400 $\mu\text{g mL}^{-1}$ G418 and their resistance to 5-FOA (0.1% w/v). Haploid clones were isolated from the engineered diploid strains using the SGA selection protocol as described previously [78]. The correct insertions were confirmed by PCR of genomic DNA of the *NUP82* locus to create strains YPAR0139, YPAR0143, YPAR0141 and YPAR0142. A PCR cassette was used to disrupt *HIS3* in YPAR0139, YPAR0143, YPAR0141 and

YPAR0142, clones were selected for their ability to grow on a medium containing hygromycin and inability to grow on a medium lacking histidine to produce strains YPAR0143, YPAR0145, YPAR0147 and YPAR0149.

Splicing of the ACT1 intron and insertion of an artificial intron within the GFP gene

Plasmids pPAR063 and pPAR061 were used to produce *GFP* transcripts containing either the *ACT1* intron (ACT1i) or an artificial intron (AI) [114], respectively, by induction from a galactose inducible promoter. Cultures were grown to mid-log phase in liquid culture with raffinose as the sole carbon source. At OD₆₀₀ of ~1, galactose or dextrose was added to a final concentration of 2% and the cultures grown at 30°C for 2 h. Total RNA was extracted from these cultures (~2 x 10⁷ cells) using the RNeasy RNA extraction kit (Qiagen). 5 µg of RNA was treated with 1 U of DNase I at 37°C for 10 min before heat inactivation at 75°C for 10 min. RNA samples were then subject to two-step RT-PCR using Superscript III with the *GFP*-specific primers:

5'-AAGCTGACCCTGAAGTTCATCTGC-3' and
5'-CGTTGTGGCTGTTGTAGTTGTACTCC-3'.

Ty1 mobility assays

Yeast strains to be assayed for their ability to support Ty1 mobility were transformed with either pPAR078 (GFP flow cytometry method), pGTy1(*HIS3*(AI)) [73] or pBDG606 [51]. To detect Ty1 mobility, single colonies from *S. cerevisiae* transformed with each plasmid were isolated for each experiment. Each experiment was performed at least three times. Colonies were first grown for 24 h in 2 mL raffinose -uracil complete medium at 30°C with agitation. 1 x 10⁵ cells from the saturated cultures were used to inoculate 15 mL of -uracil complete medium with either 2% galactose or 2% raffinose with 0.02% galactose, followed by growth for 5 days at room temperature with agitation. For pGTy1(*HIS3*(AI)) or pBDG606: Cultures were serially diluted and plated onto a -uracil complete medium and a -uracil -histidine complete medium, both with dextrose as a carbon source. Colonies were counted after 2 days growth at 30°C and the percentage mobility was calculated. For pPAR078: Cultures were diluted and allowed to reach early log phase growth (OD₆₀₀ ~0.05) before the addition of CuSO₄ to a final concentration of 0.5 mM. Cultures were grown for 9 h at 30°C before assaying for the presence of live, GFP positive cells using a BD LSRII Fortessa flow cytometer (San Jose, CA) running FACS-Diva software (v6.1.3). GFP excitation was observed with a blue, 488 nm laser, while GFP emission was collected using 530/30 nm band pass filter and 502 nm long pass filter. Propidium iodide (PI) excitation was observed with a yellow-green, 561 nm laser, while PI emission was collected using 660/20 nm band pass filter and a 635 nm long pass filter. 100,000 gated events were collected using a forward scatter vs side scatter dot plot, with forward scatter showing relative particle size and side scatter showing internal complexity. All subsequent plots were generated from this gated population. Live cells were gated by staining cell populations with PI (final concentration 0.1 µg mL⁻¹) and GFP positive populations were gated by comparison with GFP negative populations of cells. Analysis of flow cytometry data was performed using FlowJo version 9.7.6.

Ty3 mobility assay

For quantification of Ty3 mobility, yeast cells were transformed with pPS3858, a *URA3* marked galactose inducible Ty3-*HIS3* [70,74–76]. The *HIS3* gene is located at the end of *POL* and is anti-sense to Ty3, except for an artificial intron which is sense. The sense intron prevents production of His3p until after the full-length Ty3 RNA is transcribed, spliced, reverse transcribed

and integrated into the genome. Colony transformants were selected on a synthetic medium with 2% glucose (SD) complete with amino acids but lacking uracil. Single colonies were inoculated into 2 mL of synthetic raffinose (–uracil) and grown for 24 h. Cultures were then brought to 5 mL and grown for ~8 h, after which OD₆₀₀ was measured and cultures were diluted back to an OD₆₀₀ of ~0.02 in 4.5 mL and grown overnight. The following morning, 500 µL of 20% galactose (2% final) was added to induce Ty3 expression; after 8 h of induction cultures were pelleted, washed in SD medium, serially diluted, and plated on both SD plates lacking histidine for growth of transposed cells and also YPD plates to determine total live cell counts.

Nup82p^{DFY-LILF} expression assays

Plasmids constitutively expressing either Nup82p^{DFY-LILF}, Nup82p truncation mutants or Met17p were introduced into a strain containing a Ty1 mobility reporter plasmid. Mobility assays were carried out as previously described above, but with the use of a double-dropout complete medium to maintain both episomal vectors.

Western blotting of Nup82p^{DFY-LILF} and truncation mutants

Yeast lysates were prepared from 5 mL of stationary phase culture grown for 16 h in a yeast complete medium –leucine –tryptophan. Cell pellets were washed with 1 mL of chilled 25 mM Tris-HCl (pH 7.0), 10 mM sodium azide before incubation at 100°C for 3 min. 50 µL of SDS sample loading buffer (100 mM Tris-HCl, 5% SDS, 10% glycerol, 0.1% bromophenol blue, 2% β-mercaptoethanol, pH 6.8) was added to the boiled pellet with 200 µL of acid-washed glass beads (0.5 mm). Samples were vortexed for 10 min to disrupt yeast cells before the addition of another 80 µL of SDS sample loading buffer. Glass beads were pelleted by centrifugation (1500 × g, 2 min). 30 µL of each sample was loaded directly onto a precast Tris-glycine 10% SDS-PAGE gel (Biorad). Flag-tagged *NUP82* mutants were detected via Western blot using a 1:4000 dilution of a primary mouse monoclonal anti-flag (Syd Labs #M20008). Secondary detection was carried out using a 1:2000 dilution of a goat anti-mouse horseradish peroxidase conjugated antibody (Thermo #32430).

Nuclear import assays

A LexA-MBP-GAL4(AD) fusion protein with or without an SV40 nuclear localization signal [71] was used to measure the efficiency of nuclear import within *S. cerevisiae* L40 or BY4741. 5 mL of a glucose-supplemented synthetic complete medium lacking the appropriate amino acid and grown overnight at 30°C with agitation. Cells were collected by centrifugation at 4000 × g for 30 seconds and the cell pellets suspended in 750 µL of ice-cold ddH₂O. Washed cells were again collected by centrifugation (13,000 × g for 30 seconds), and soluble proteins extracted by Y-PER buffer as per manufacturer's instructions (Thermo). The lysate was assayed for β-galactosidase activity as described previously [118].

Fluorescence microscopy

The steady-state import of GFP-NLS was monitored within BY4741 transformed with pEB0836 as described previously [72].

Detection of Ty1 genomic integrations by Southern blotting

The detection of the integration of Ty1 containing *GFP* by Southern blotting was performed as previously described [73], in the various *NUP84*-complemented or deletion strains of *S.*

cerevisiae. Total DNA was extracted from cell cultures using phenol:chloroform and ethanol precipitation, after 5 days of induction, as described in the Ty1 mobility assay protocol above. Southern blotting was carried out after agarose gel electrophoresis, as described previously [119], using Hybond-XL membranes (GE healthcare).

Promoter activity assay

To assay the activity of the *GAL1* promoter we expressed *GFP* under the control of the *GAL1* promoter and monitored the increase in the mean fluorescent intensity (MFI) compared to uninduced control cells. Cells were grown overnight to saturation at 30°C (CM -uracil, 2% raffinose) before being used to seed a 10 mL culture that was grown to log phase (OD₆₀₀ 0.1–0.5). Each 10 mL log phase culture was divided into two 5 mL cultures, supplemented with either 2% galactose or dextrose (final concentration) and grown for 6 h. Cultures were assayed for GFP fluorescence by flow cytometry using the same instrumentation as described above. The activity of the *CUP1* promoter was assayed by analyzing MFI data derived from Ty1-GFP mobility assays.

Supporting information

S1 Fig. Nucleoporins and karyopherins are important for Ty mobility, but karyopherins are not evolving rapidly. (A) A summary of whole genome studies that have identified nucleoporins and karyopherins important for Ty1 and Ty3 mobility [51–57]. (B) Results from PAML analysis surveying karyopherins for signatures of positive selection, comparing a codon model of purifying selection (M7) to a codon model of positive selection (M8). No karyopherins had a $p < 0.05$. (TIF)

S2 Fig. Ty1 mobility is generally reduced when assayed from a single copy reporter plasmid in *S. cerevisiae* expressing orthologous *NUP84*. (A) Relative mobility of Ty1 was assayed with a single copy plasmid within strains complemented with *NUP84* from different *Saccharomyces* species using the auxotrophic marker *HIS3*. Ty1 transcription was initiated by high (2%) or low (0.02%) concentrations of galactose (B) Averaged percentage of cells that scored positive for Ty1 mobility (Y axis) comparing low- (centromeric; CEN) and high- (2-micron plasmid; 2 μ m) copy number plasmids with the expression of Ty1 driven by high or low levels of expression via the *GAL1* promoter (error bars: standard error, $n > 4$). For the GFP assay this was calculated as the overall percentage of GFP +ve cells in the total population. For the CEN plasmid, this was calculated as the percentage of cells that could grow on a complete medium lacking histidine. (TIF)

S3 Fig. The construction of *S. cerevisiae* strains expressing heterospecific *NUP82*. The *KANMX6* gene within a diploid strain of *S. cerevisiae* heterozygous for *KANMX6* at one *NUP82* locus (A) was replaced with the *URA3* gene from *K. lactis* flanked by *SceI* sites (B–C). *SceI* restriction endonuclease was used to create double-stranded DNA breaks at the *URA3*-containing *NUP82* locus, which was simultaneously repaired by a PCR-derived cassette encoding heterospecific *NUP82* and *KANMX6* (D–E). Haploid clones were isolated using the SGA selection protocol [78] (F–G). (TIF)

S4 Fig. The evolution of *NUP82* does not impact GFP production from different promoters. The effect of *NUP84* complementation on the ability of *S. cerevisiae* to express GFP from

the promoters used in our Ty1 *GFP*-based reporter (*GAL1* or *CUP1* promoters).
(TIF)

S1 File. Evolutionary analysis of genes involved nucleocytoplasmic transport. This spreadsheet summarizes the results from all of the evolutionary analyses that were performed.
(XLSX)

S2 File. Plasmid sequences. This file contains sequences of plasmids constructed as part of this study.
(TXT)

S1 Table. Plasmid sequences. A table containing the names and descriptions of all plasmids used in this study and their origins.
(DOCX)

S2 Table. Yeast strains. A table containing the names and descriptions of all yeast strains used in this study and their origins.
(DOCX)

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