Silent but Not Static: Accelerated Base-Pair Substitution in Silenced Chromatin of Budding Yeasts

Leonid Teytelman1, Michael B. Eisen1,2,3, Jasper Rine1,2

1 Department of Molecular & Cell Biology, University of California Berkeley, Berkeley, California, United States of America, 2 California Institute for Quantitative Biosciences, Berkeley, California, United States of America, 3 Center for Integrative Genomics, University of California Berkeley, Berkeley, California, United States of America

Abstract

Subtelomeric DNA in budding yeasts, like metazoan heterochromatin, is gene poor, repetitive, transiently silenced, and highly dynamic. The rapid evolution of subtelomeric regions is commonly thought to arise from transposon activity and increased recombination between repetitive elements. However, we found evidence of an additional factor in this diversification. We observed a surprising level of nucleotide divergence in transcriptionally silenced regions in inter-species comparisons of Saccharomyces yeasts. Likewise, intra-species analysis of polymorphisms also revealed increased SNP frequencies in both intergenic and synonymous coding positions of silenced DNA. This analysis suggested that silenced DNA in Saccharomyces cerevisiae and closely related species had increased single base-pair substitution that was likely due to the effects of the silencing machinery on DNA replication or repair.

Introduction

The ends of chromosomes in yeasts, vertebrates, Drosophila, and eukaryotic pathogens such as Plasmodium falciparum diverge more rapidly than the rest of their genomes [1]. In budding yeasts of the genus Saccharomyces, chromosome ends contain a high density of repeated sequences and relatively few genes; they are more diverged between species than any other portions of the genomes, and are highly variable within species [2,3]. The accelerated diversification of subtelomeric DNA is commonly attributed to the presence of transposons and the repetitive nature of these regions, as both contribute to recombination between different chromosome ends [4,5]. However, subtelomeric regions in yeasts are also silenced, analogously to metazoan heterochromatin [6], raising the possibility that the formation and maintenance of a silenced chromatin state contribute to the observed rapid evolution.

In S. cerevisiae, the best characterized silenced regions are the HML and HMR transcriptionally inactive mating loci of chromosome III. They contain non-expressed copies of the MATα and MATα mating-type genes. During mating type interconversion, HML or HMR is copied into the MAT locus, also on chromosome III, where the resident allele is transcribed. Since haploid cells that express both MATα and MATα behave as non-mating diploids, it is crucial that HML and HMR are silenced. This is achieved through the E and I silencers that flank both of the silenced loci (Figure 1) and recruit Silent Information Regulator (Sir) proteins which then spread throughout the regions. The Sir proteins bind to and deacetylate the tails of histones H3 and H4, leading to silencing of HML and HMR [7].

The Sir2/Sir3/Sir4 protein complex that is responsible for HML and HMR silencing also binds to subtelomeric regions of S. cerevisiae chromosomes [8]. In contrast to the strong and robust silencing of HML and HMR, subtelomeric silencing is weaker [9]. Nevertheless, native telomere-proximal genes and reporter genes inserted near telomeres are reliably silenced [10–13].

The Saccharomyces sensu stricto species (S. paradoxus, S. mikatae, S. kudriavzevii, S. bayanus) genome sequences are sufficiently closely related to allow identification of conserved regulatory sequences [14]. Essentially all S. cerevisiae protein-coding genes are found in these other species, and most orthologous intergenic regions in the sensu strico yeasts can be readily aligned [2,15]. However, in analyzing the evolution of the HML and HMR silencers, we discovered a surprising lack of DNA conservation in all four flanking regions, motivating an in-depth exploration of the evolution of silenced regions within and between these yeast species. Our observations suggested an additional force in the shaping of these regions.

Results

Lack of Cross-Species Conservation in Sequences Flanking HML and HMR

To identify the E and I silencers in the sensu stricto species, we searched for peaks of conservation in multiple sequence alignments. For both of the S. cerevisiae HML and HMR, we identified contigs in the sequenced sensu stricto species that contained a part of the locus and the adjacent gene. The right side of HMR was misassembled in S. paradoxus with two disjointed contigs with incorrect inverted ends, so we resequenced and assembled the region (GenBank EU597267). HML and HMR were conserved across all five species with clearly conserved orthologs of the neighboring genes (Table S1). However, unlike most intergenic sequences in the genome, the regions around HML and HMR were too diverged to allow multiple alignments. Moreover, local
Author Summary

Many plants, fungi, pathogens, and animals have chromosome regions that are silenced. Special proteins change the chromosomal structure in these domains, turning genes off or lowering their expression levels. We found an increased frequency of DNA mutations in these silenced regions of closely related yeasts. This increase is likely due to silencing proteins interfering with DNA repair or replication. Accurate replication of genetic information with minimal mutations is usually critical for the survival and fitness of an organism; however, there are examples where a high mutation rate is beneficial. The silenced regions of chromosomes are often associated with virus-like transposable elements, and with genes that are important in responding to environmental changes. Hence, it is possible that elevated DNA mutations in silenced regions contribute to genome defense against transposable elements or increased genetic diversity to cope with variation in surrounding conditions.

pairwise alignments of these flanking sequences between any of the ten species pairs were also unexpectedly dissimilar. The best pairwise alignments were between the two closest species S. cerevisiae and S. paradoxus, but instead of the genome-wide average of 80% identity for orthologous intergenic regions, the percent identities were: 46% left of HML, 55% right of HML, 52% left of HMR, 45% right of HMR. These alignments were almost as dissimilar as if the sequences were unrelated; 1000 random equal-length sequences with identical base composition that we dissimilar as if the sequences were unrelated; 1000 random equal-length sequences with identical base composition that we generated had an averaged local pairwise similarity of 45%. BLAST-based comparisons also did not reveal matches for the generated had an averaged local pairwise similarity of 45%. BLAST-based comparisons also did not reveal matches for the sequences between HML or HMR and the nearest flanking genes, ruling out local inversions and rearrangements (Figure 2).

Translocations or transpositions could, in principle, have lead to poor alignments across the species in the HML and HMR flanking regions. In such a case, sequence searches from one species would be expected to produce matches in non-syntenic positions of other species. However, BLAST searches with the diverged intergenic segments around HML and HMR from each of the five species against the assembled genomes of the other species did not produce significant BLAST results outside of the syntentic contigs. The only exceptions were the S. cerevisiae to S. paradoxus matches in repetitive DNA (Figure S1); however these likely reflect homogenization of these repeated sequences by gene conversion rather than functional conservation [16–18]. We also excluded the possibility that systematic misassembly occurred in these regions in the sensu stricto by performing BLAST searches against the unassembled traces of each species. Therefore, sequence assembly issues and rearrangements did not explain the poor alignments of DNA sequences flanking HML and HMR.

Conservation of Silencer Sequences within Highly Diverged Intergenic DNA

We determined that the flanking sequences in the five species were indeed orthologous by analyzing conservation of the silencers that have been identified in S. cerevisiae. In three of the four cases (HMR-E, HMR-I, HML-I), there was clear conservation of the known functional binding sites in the silencers, despite the low sequence similarity throughout the intergenic regions. To the right of HML, an Abf1 binding site was present 319-321 base pairs past the HMLa1 stop codon in all five species. At HMR-I, the sequence of the Rap1 and Abf1 binding sites, their orientation, distance to HMR, and spacing between the binding sites were conserved between S. cerevisiae and S. paradoxus. Similarly, the Abf1 and Rap1 binding sites in HMR-E were conserved in all five species, with virtually the same spacing between the sites (39–43 bp), and the distance to HMR was identical in S. paradoxus and S. cerevisiae (Figure 3, Figure S2).

Functional Conservation of the HMR-E Silencer between S. cerevisiae and S. bayanus

To test if the observed sequence conservation reflected functional conservation, we deleted a 140-bp fragment containing known Abf1p and Rap1p binding sites from the presumptive HMR-E in haploid S. bayanus. The deletion abolished silencing at the HMR locus to the same extent as did deletion of the SIR2 gene (Figure 4). This experiment, together with the in silico observations of the conservation of binding sites and silencer architectures in the HML and HMR silencers, established that the regions from the five species were orthologous and suggested that the DNA flanking the HMR loci evolved more rapidly than other intergenic DNA.

Subtelomeric Intergenic DNA Overrepresented in Highly Diverged Regions

Intrigued by the unusual divergence around HML and HMR, we sought to determine if other silenced regions were enriched for diverged sequences. We searched all 6,217 S. cerevisiae intergenic regions for DNA sequences without significant matches to any of the other sensu stricto genomes (Table S2). In subtelomeric regions, defined as the 50 kb internal to each telomere [2], there was an unmistakable enrichment of these non-conserved intergenic sequences. Of the 344 S. cerevisiae intergenic regions with no...
matches to the sensu stricto, over 40% were subtelomeric, even though subtelomeric DNA constituted less than 20% of the total analyzed S. cerevisiae intergenic DNA ($p<10^{-10}$ by $\chi^2$-statistic).

In principle, unequal recombination between repetitive elements and transposon activity might have caused sufficient insertions and deletions to result in segments of subtelomeric DNA in S. cerevisiae that lacked counterparts in S. paradoxus. Therefore we counted intergenic regions with detectable homology but less than 70% identity between the two species (Table S3). If the enrichment of unique sequences in subtelomeric regions were due to insertions and deletions, we would not expect to also see a subtelomeric enrichment of low-identity regions. However, similarly to the excess of unmatched segments, 12% of intergenic subtelomeric DNA had low-identity matches between S. cerevisiae and S. paradoxus, compared to 7% in the rest of the genome ($p<10^{-10}$ by $\chi^2$-statistic). Therefore, an excess of insertions and deletions could not be the sole reason for the enrichment of diverged intergenic sequences in subtelomeric regions.

Unmatched and poorly conserved subtelomeric intergenic regions were found on all chromosomes (Table S2, Table S3). Therefore, the higher-than-expected divergence was not unique to HML, HMR, or the chromosome that bears them, but was a general phenomenon common to silenced regions.

High SNP Frequency in Sequences Flanking HML and HMR and in Subtelomeric Intergenic Regions

If rapid divergence were an inherent property of silenced DNA, more intra-species polymorphisms in these regions would also be expected. We measured genome-wide average intergenic SNP frequencies in S. cerevisiae and S. paradoxus [19] and compared them to the frequencies in sequences flanking HML and HMR. Although the HML and HMR loci, per se, and the four neighboring genes exhibited

Figure 2. Lack of conservation in HML and HMR flanking intergenic regions. The results from the BLAST searches with S. cerevisiae HML and HMR and surrounding sequence against corresponding syntenic S. paradoxus contigs are shown with percent identity plotted for 200-bp windows. Genes are annotated on the x-axis. Segments without significant BLAST matches are shaded.

doi:10.1371/journal.pgen.1000247.g002

Figure 3. Conservation of the HMR-E silencer in five sensu stricto species. Multiple alignment of the putative HMR-E silencer in the five sensu stricto species. There was strong conservation among all of the species of the Rap1 and Abf1 binding sites (shaded) and the spacing between them, with diverged intervening sequence from the Rap1 site to the Abf1 site. Similarly, even though the distance from the Abf1 site to HMR was identical in S. cerevisiae and S. paradoxus, DNA-level pairwise alignment of the region gave only 55% identity (Figure S1).

doi:10.1371/journal.pgen.1000247.g003
SNP frequencies typical of genome-wide averages, the intergenic silenced DNA around HML or HMR had SNP frequencies two to three times higher than average in both species (Figure 5).

A similar pattern of SNP frequencies to that observed at the HML loci was also detected for telomere-proximal intergenic regions among S. cerevisiae isolates. To avoid counting polymorphisms arising from recombination between repetitive DNA sequences, only SNPs in single-copy intergenic regions were considered. SNPs were significantly more frequent in subtelomeric regions, within 0–20 and 20–40 kilobases of telomere edges, than in the rest of the genome (Figure 6, upper panel). The subtelomeric regions were the only ones that deviated strongly from the genome-wide averages.

High SNP Frequency in Synonymous Codons of Subtelomeric Genes

Increased polymorphisms in subtelomeric and HML and HMR-flanking DNA could result from accelerated base-pair substitutions or from decreased selective constraint on these regions. To distinguish these two possibilities, we analyzed polymorphisms in synonymous positions of codons. If subtelomeric intergenic regions were diverging faster than non-subtelomeric ones because of lower functional constraint, then higher SNP frequencies would be expected for the intergenic but not for synonymous coding positions of subtelomeric DNA.

We counted SNPs at fourfold-degenerate synonymous sites of single-copy genes in S. cerevisiae; dubious genes were excluded. Synonymous SNP frequencies in subtelomeric genes were significantly elevated, compared to the rest of the genome, and the level of increase was similar in the synonymous coding and in intergenic positions (Figure 6, lower panel). For the analyzed subtelomeric and non-subtelomeric genes, there was no significant difference in protein-level conservation of orthologs between S. cerevisiae and S. paradoxus (Wilcoxon–Mann–Whitney p = 0.10) (Figure S3). For the codons of the four genes flanking HML and HMR in S. cerevisiae, the fourfold-degenerate synonymous SNP frequency was also elevated compared to the genome-wide average (7% versus 4.4%), however due to the small number of total synonymous sites, the difference was less statistically impressive (p = 0.01 by χ²-statistic).

Presumably, fourfold-degenerate synonymous sites of similarly conserved genes are under the same selection, regardless of chromosome position. The concordance between SNP frequencies in intergenic regions and in synonymous codon positions in functional genes implied that the higher SNP frequency closer to chromosome ends resulted from hyperdivergence rather than relaxed selective constraint.

Transcription-Coupled Repair Did Not Explain Elevated Subtelomeric Substitution

Transcription-coupled repair is a type of the general nucleotide excision repair that targets repair machinery to highly transcribed genes [20]. One possible model is that silenced DNA, by virtue of its lack of expression, is deficient in transcription-coupled repair, resulting in increased substitutions. We tested this possibility by analyzing the effect of expression on SNP frequencies for intergenic and coding regions.

A genome-wide RNA-sequencing dataset [21] was used to assign median expression level for each gene and intergenic region. The extent of expression of intergenic DNA was indistinguishable between the most telomere-proximal and non-subtelomeric regions (Figure 7A). As would be expected from the observation, there was no correlation between intergenic expression and SNP density (Figure 7B). For genes, there was a definite decrease in median expression of subtelomeric genes (Figure 7A). However, as for the intergenic regions, there was no increase in SNP frequencies for highly expressed genes (Figure 7C).

Therefore the lack of coding or non-coding correlation between expression and SNP frequencies indicated that transcription-coupled repair was not likely to have contributed to the hyperdivergence of DNA sequence in silenced regions.

Discussion

Chromosome ends vary widely among the sensu stricto species due to transposons, gene families, and other repetitive elements [2]. By focusing on orthologous sequences that flank the HML and HMR loci in these species and on unique subtelomeric DNA, we...
Accelerated Mutation Frequency in Silenced DNA

Intergenic regions

Synonymous codons
identified an additional contribution to diversification of these regions: increased base-pair substitutions.

The data in this paper were based upon SNP frequencies, which reflect the combined effect of the rate of nucleotide change and repair, and the strength of selection. Because the elevated SNP frequency was also found in silenced regions in synonymous coding positions, the most parsimonious view was that selection had little if any impact on these frequencies. Therefore, we inferred that the increased SNP frequency in silenced chromatin reflected an increased mutation rate; whether that increased rate resulted from increased rates of substitution or repair, or both, could not, at present, be determined.

Our analysis of inter- and intra-species variation detected a clear and compelling correlation between Sir-silenced regions and those that exhibited hyperdivergence. In S. cerevisiae, the increase in SNP frequencies was higher in constitutively silenced HML and HMR regions than in the transiently silenced subtelomeric DNA. We considered a myriad of other explanations including proximity to tRNAs, transposons, LTRs, and autonomous replicating sequences and also base composition; however, none of these genomic features explained the dramatic increase in divergence within subtelomeres and in regions flanking HML and HMR. Because silencing can interfere with DNA repair, Sir-based silencing appeared to be the most likely mechanism for this rapid sequence diversification. DNA at the expressed MAT locus is repaired 2.5 times faster than identical DNA at the silenced HML locus [22], and silencing interferes with both photolyase and nucleotide excision repair pathways at a subtelomeric position, independently of transcription [23]. Although Sir-based inhibition of repair was an adequate explanation of these data, we could not exclude the possibility that silenced chromatin may have intrinsically reduced replication fidelity. We considered other possible explanations, of which transcription-coupled repair seemed most plausible, since it should be rendered less useful for genes subject to silencing. However, upon genome-wide analysis, we found no correlation between the level of expression and the frequency of SNPs. Hence, transcription-coupled repair was an unlikely explanation for the increased mutation rate in silenced regions of the genome.

In principle, it should be possible to test whether Sir-based silencing were responsible for the rapid diversification of sequences near and within silenced regions by evolving Sir+ and Sir− strains over a sufficiently long time, and then sequencing the genomes. However, our best estimate of the time that would be required suggested this approach was impractical. There is little doubt that the URA3 gene, if inserted in silenced regions, could be used to detect a higher frequency of ura3 mutations in silenced versus non-silenced regions of the genome. However, the phenotypic lag introduced by the higher expression level of URA3 in the Sir− cells would give the expected correlation of Sir genotype to mutation rate, but for the wrong reason.

Regardless of the underlying mechanism, the potential benefit or detriment to the cell of elevated substitutions in subtelomeres is an intriguing question. Subtelomeric regions are gene poor; therefore the cost of increased mutation rate in these regions might merely be tolerated by the yeasts. However, certain characteristics of heterochromatin in many different organisms and of sub-

telomeric DNA in yeasts and eukaryotic pathogens raised the possibility that an increased mutation rate may have selective advantage. Heterochromatin in many fungi, animals, and plants commonly contains transposable elements [24,25]. In budding yeasts, silenced DNA is a hotspot for Ty5 retrotransposon insertion [16], and the Sir4 silencing protein directly interacts with the integrase of Ty5, targeting it to silenced DNA [26]. Silenced chromatin could serve as a decoy to attract an invading transposon to that portion of the genome where its expression would be inhibited, while increased rates of substitution would help to inactivate the newly incorporated transposon [27].

An alternate hypothesis for a beneficial role of hyperdivergence is inhibition of deleterious recombination. Ectopic recombination between repetitive subtelomeric DNA sequences destabilizes the genome. Of the 19 reciprocal translocations identified in the Saccharomyces species, 11 are in subtelomeric regions [2]. Subtelomeric sequences may also promote proper segregation of chromosomes by decreasing meiotic recombination in chromosome ends [28–30]. Increased divergence and subsequent reduction in sequence identity would be expected to lower both ectopic recombination between subtelomeric repeat elements and meiotic crossovers in chromosome ends.

It is also possible that residence within hyperdivergent regions may facilitate diversity of certain classes of genes. In S. cerevisiae, many of the subtelomeric genes play a role in adapting to changes in environmental conditions [31,32]. Antigenic variation of most eukaryotic pathogenic parasites relies on subtelomERICally positioned genes [33]. If silencing-based hypersubstitution also occurs in these pathogens, it may aid in host immune evasion. More broadly, transient subtelomeric silencing combined with accelerated DNA evolution may increase phenotypic diversity, allowing organisms to cope with environmental changes. Of course, increased diversity in perpetually silenced genes would have questionable evolutionary value. However, most subtelomeric genes are only partially silenced, with the level of silencing both variable on a cell-to-cell basis and heritable through multiple cell divisions. The striking exception to hypermutation in heterochromatic genes in our data were the HML and HMR loci themselves. Because these loci are in frequent recombinational communication with the MAT locus, the powerful selection exerted on MAT was presumably the force that, through recombination, removed the variation in HML and HMR that would be expected, based upon our hypothesis.

Two recent studies indicate an elevated substitution rate in X chromosome subtelomeric regions and Troponin C gene family members of Drosophila melanogaster [34,35]. Our study established the generality of this effect across taxa, extended it to the full genome analysis, and excluded all proposed mechanisms except for elevated mutation in silenced regions. Given the conservation of heterochromatic hyperdivergence across taxa, it is presumably beneficial and it may be that increased base-pair substitutions contribute simultaneously to genome stability and to adaptive evolution.

Materials and Methods

Yeast Strains

All of the yeast strains used in this study are listed in Table 1.
The URA3 gene was replaced in S. bayanus strain JRY7880 with the hph gene (EUROSCARF plasmid pAG32, [36]), producing the ura3-::hph strain (JRY8772). The resulting strain was crossed to JRY7890 to give JRY8774 and JRY8775 (from two different tetrads). Next, the 138-bp fragment of the putative S. bayanus HMR-E, containing matches to the Abf1 and Rap1 binding sites, was deleted through transformation and homologous recombination with a loxP-K. lactis URA3-loxP construct (EUROSCARF plasmid pUG72, [37]). In the resulting strains (JRY8781 and JRY8782), the K. lactis URA3 sequence was excised by expressing the Cre recombinase (EUROSCARF plasmid pSH62, [37]). The hmr-e deletion in the final strains (JRY8785 and JRY8786) was confirmed by sequencing. As a result of these manipulations, the original 138-bp putative HMR-E sequence was replaced with 134-bp sequence from pUG72, containing one copy of a loxP site and flanking nucleotides from the vector (hmr-e::loxP).

S. Bayanus HMR-E Manipulation

The URA3 gene was replaced in S. bayanus strain JRY7880 with the hph gene (EUROSCARF plasmid pAG32, [36]), producing the ura3-::hph strain (JRY8772). The resulting strain was crossed to JRY7890 to give JRY8774 and JRY8775 (from two different tetrads). Next, the 138-bp fragment of the putative S. bayanus HMR-E, containing matches to the Abf1 and Rap1 binding sites, was deleted through transformation and homologous recombination with a loxP-K. lactis URA3-loxP construct (EUROSCARF plasmid pUG72, [37]). In the resulting strains (JRY8781 and JRY8782), the K. lactis URA3 sequence was excised by expressing the Cre recombinase (EUROSCARF plasmid pSH62, [37]). The hmr-e deletion in the final strains (JRY8785 and JRY8786) was confirmed by sequencing. As a result of these manipulations, the original 138-bp putative HMR-E sequence was replaced with 134-bp sequence from pUG72, containing one copy of a loxP site and flanking nucleotides from the vector (hmr-e::loxP).

Silencing Assay

The phenotypic consequence of the hmr-e deletion in S. bayanus was assayed by comparing mating ability of the hmr-e::loxP-MATa strains (JRY8785 and JRY8786) to the parental HMR-E strains (JRY8781, JRY8782). The S. bayanus strains were patched onto synthetic dextrose minimal medium plates [38], overlapping patches of S. cerevisiae Matα mating tester (JRY2726). Only diploid hybrids resulting from mating would be histidine prototrophs and able to grow. The disruption in HMR silencing changed the MATa mating type to the non-mating phenotype of MATa/MATα diploids, interfering with the haploid’s ability to mate with the S. cerevisiae Matα tester.

Sequencing S. paradoxus DNA Flanking the Right Side of HMR

S. paradoxus genomic DNA was isolated from JRY7910 using the Qiagen Miniprep kit. 5 kb fragment from HMRa1 to GIT1 was amplified with LongTemplate DNA polymerase PCR (forward primer: GTCACCTCAAAGTTAGAGTTTGGG; reverse prim-
er: TTATTAGCAGTGAGCGTCAGCCA). 12 primer sets were used in sequencing reactions to produce overlapping fragments along the 5 kb sequence, and the fragments were subsequently manually assembled based on overlap and deposited in GenBank (EU509726).

Pairwise Alignments

Multiple alignments were made using the ClustalW program [39]. Local pairwise Smith-Waterman alignments [40] between S. cerevisiae and S. paradoxus sequences flanking HML and HMR were performed using the EMBOSS “water” program [41] with DNA-matrix, gap-open penalty of 9 and gap-extension penalty of 1. The flanking regions to the left and right of the HML and HMR loci were based on the annotations in Table S1, using all intergenic regions from the edge of each flanking gene to the nearest HML/HMR edge. Estimation of percent identity in local pairwise alignments of unrelated DNA sequence was based on 1000 alignments between 4,000 base-pair, randomly generated DNA sequences with AT content, matching that of the left side of HMR (67%).

BLAST Searches

All BLAST searches were performed using NCBI BLAST [42] without repeat masking (∼F, and with mismatch penalty of −1 (−q −1). For HML/HMR BLASTs, e-value cutoff was set at 10−3; for all other searches, the cutoff was 10−2. The “blastp” program was used for S. cerevisiae and S. paradoxus orthologous protein comparisons; and the “blastn” program was used for all other intergenic and coding DNA BLASTs.

Subtelomeric versus Non-Subtelomeric Intergenic Conservation

Intergenic regions of S. cerevisiae were defined as sequences between transcript edges of all SGD-annotated genes, including uncharacterized, dubious, and coding regions. Transcript edges were defined using the annotations from the RNA-sequencing dataset [21], to exclude 5’ and 3’ untranslated regions from the intergenic sequence. Overlapping BLAST matches to S. paradoxus were merged into contiguous blocks, regardless of synteny. S. cerevisiae intergenic sequences 250 base-pairs or longer without BLAST results were considered unmatched. In analysis of poorly conserved intergenic DNA, BLAST matches with less than 70% identity were compared to matches with greater than 70% identity.

SNP Analysis

S. cerevisiae and S. paradoxus SNP positions were downloaded from http://www.sanger.ac.uk/Teams/Team1/durbin/sgrp [19]. SNPs within 50 kilobases of chromosome ends were counted as subtelomeric, and those at greater distances as non-subtelomeric. Single-copy genes and intergenic DNA were defined as S. cerevisiae sequences that produced only a single significant BLAST match to themselves. If any part of an intergenic region or a gene had additional BLAST matches, the whole region or gene was excluded from the SNP analysis. Genes classified as “dubious” in the Saccharomyces Genome Database were not considered.

Expression Analysis

Expression levels were obtained from the genome-wide RNA-sequencing dataset [21]. For each transcript and intergenic region, expression level was defined as the median of all the mapped RNA sequencing reads from that segment. SNP frequencies, as described above for the intergenic and synonymous coding regions, were graphed against the respective expression levels, as indicated on the x-axes of Figure 7B and Figure 7C.

Ortholog Conservation between S. cerevisiae and S. paradoxus

S. paradoxus orthologs of S. cerevisiae genes were determined based on best-reciprocal BLAST matches. All possible peptide sequences longer than 50 residues were extracted from six-frame translation of the S. paradoxus genome. Verified and uncharacterized SGD-annotated S. cerevisiae proteins were BLASTed against all the potential S. paradoxus peptides. For each S. cerevisiae protein (Xc), the best S. paradoxus match (Xp) was then BLASTed back against all S. cerevisiae proteins, and if the best match for Xp was also Xc, the pair was defined as orthologous. For the genes used in SNP analysis (non-dubious and single-copy in S. cerevisiae), distribution of protein percent identity of subtelomeric S. cerevisiae—S. paradoxus orthologs was compared to orthologs positioned greater than 50 kilobases from chromosome ends in S. cerevisiae.

Statistical Analyses

All statistical tests were performed using R [43].

Supporting Information

Figure S1 Lack of conservation in HML and HMR flanking intergenic regions. BLAST searches with S. cerevisiae HML and HMR and surrounding sequence against S. paradoxus. Upper panel shows BLAST results against syntenic S. paradoxus contigs that contain HMR and HML. Lower panel displays BLAST results with the same S. cerevisiae query sequence against the entire genome of S. paradoxus. Percent identity is plotted in 200-bp windows. Genes and mating loci are annotated on the x-axis. Segments without significant BLAST matches are shaded. Additional matches around HMR from searches against all of S. paradoxus were mostly due to repeated sequences, as can be seen from the stacking of matches (compare upper and lower panels of HMR).

Found at: doi:10.1371/journal.pgen.1000247.s001 (1.55 MB TIF)

Figure S2 S. cerevisiae and S. paradoxus lack of sequence conservation between HMR-E Abf1 binding site and HMR. Pairwise global alignment (Needleman-Wunsch) of DNA sequence between the HMR-E Abf1 binding site and the HMR edge, comparing S. cerevisiae to S. paradoxus. Length of the intervening sequence between HMR-E and HMR was identical in both species, but sequence conservation itself was poor.

Found at: doi:10.1371/journal.pgen.1000247.s002 (0.00 MB TXT)

Figure S3 Similar conservation of subtelomeric and non-subtelomeric genes between S. cerevisiae and S. paradoxus. Distributions of protein-level percent identities between S. cerevisiae and S. paradoxus orthologous genes, comparing subtelomeric versus non-subtelomeric genes. No significant difference in cross-species conservation of subtelomeric versus non-subtelomeric orthologs was evident (Wilcoxon-Mann-Whitney p = 0.10).

Found at: doi:10.1371/journal.pgen.1000247.s003 (1.16 MB TIF)

Table S1 Annotation of the sensu stricto contigs corresponding to S. cerevisiae HML and HMR loci.

Found at: doi:10.1371/journal.pgen.1000247.s004 (0.01 MB XLS)

Table S2 S. cerevisiae intergenic regions with no BLAST matches in sensu stricto species.

Found at: doi:10.1371/journal.pgen.1000247.s005 (0.01 MB TXT)

Table S3 S. cerevisiae intergenic regions with less than 70% identity matches in S. paradoxus.

Found at: doi:10.1371/journal.pgen.1000247.s006 (0.02 MB TXT)
Acknowledgements

We thank Devin Scannell for discussions regarding neutral selection and Joshua Babiarz, Gary Karpen, Peter Dimitrov, and members of our labs for thoughtful comments and criticisms throughout the course of this work. We thank Jacob Mayfield, Ravi Sachidanandam, Devin Scannell, and Jason Stajich for comments on the manuscript. We also thank Ed Louis and Oliver Zill for the seston stetico strains. SNP analyses would be impossible without the pre-publication release of data by Richard Durbin and Ed Louis. The revised version of our manuscript was greatly improved thanks to the comments of Jim Haber and the anonymous reviewers.

Author Contributions

Conceived and designed the experiments: LT MBE JR. Performed the experiments: LT. Analyzed the data: LT. Wrote the paper: LT MBE JR.

References

1. Nosè J, Tomaska L, eds (2008) Origin and evolution of telomeres. Austin, TX: Landes Bioscience.
2. Kellis M, Patterson N, Endrizzi M, Birren B, Lander ES (2003) Sequencing and comparison of yeast species to identify genes and regulatory elements. Nature 423: 241–254.
3. Louis EJ (1995) The chromosome ends of Saccharomyces cerevisiae. Yeast 11: 1553–1573.
4. Linardopoulou EV, Williams EM, Fan Y, Friedman C, Young JM, et al. (2005) Human subtelomeres are hot spots of interchromosomal recombination and segmental duplication. Nature 437: 94–100.
5. Mewborn SK, Lese Martin C, Leidtter DH (2005) The dynamic nature and evolutionary history of subtelomeric and pericentromeric regions. Cytogeteng Genome Res 108: 22–25.
6. Grewe SI, Jia S (2007) Heterochromatin revisited. Nat Rev Genet 8: 35–46.
7. Rutsche LN, Kirchnauer AL, Rine J (2003) The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. Annu Rev Biochem 72: 481–516.
8. Lüb JD, Liu X, Botstein D, Brown PO (2001) Promoter-specific binding of Rap1 revealed by genome-wide maps of protein-DNA association. Nat Genet 28: 327–334.
9. Tham WH, Zakian VA (2002) Transcriptional silencing at Saccharomyces telomeres: implications for other organisms. Oncogene 21: 512–521.
10. Gotschlich DE, Aparicio OM, Billington BJ, Zakian VA (1996) Position effect at S. cerevisiae telomeres: reversible repression of Pol II transcription. Cell 63: 751–762.
11. Vega-Palas MA, Martin-Figueroa E, Florence EJ (2000) Telomeric silencing of a natural subtelomeric gene. Mol Gen Genet 259: 297–307.
12. Barton AB, Kaback DB (2006) Telomeric silencing of an open reading frame in S. cerevisiae. Genetics 171: 1089–1097.
13. Clift PF, Hillier LW, Fulton L, Graves T, Miner T, et al. (2001) Surveying genomes to identify functional elements by comparative DNA sequence analysis. Genome Res 11: 1175–1186.
14. Clifton P, Sudarsanam P, Desikan A, Fulton L, Burton P, et al. (2003) Finding functional features in Saccharomyces genomes by phylogenetic footprinting. Science 301: 71–76.
15. Kim JM, Vanguri S, Boeke JD, Gabriel A, Voskides DF (1998) Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete Saccharomyces cerevisiae genome sequence. Genome Res 8: 464–478.
16. Kejnovsky E, Holba R, Kubat Z, Widmer A, Masais GA, et al. (2007) High intrachromosomal similarity of retrotransposon long terminal repeats: evidence for homogenization by gene conversion on plant sex chromosomes? Gene 390: 92–97.
17. Hughes JF, Coffin JM (2005) Human endogenous retroviral elements as indicators of ectopic recombination events in the primate genome. Genetics 171: 1103–1119.
18. Carter D, Litt G, Moses A, Parts L, James S, et al. (2008) Population genomics of domestic and wild yeasts. Nature Precedings; hdl:10101/npre.2008.1988.1.
19. Svejstrup JQ (2002) Mechanisms of transcription-coupled DNA repair. Nat Rev Mol Cell Biol 3: 21–29.