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Genetic Basis of Hidden Phenotypic Variation Revealed by Increased Translational Readthrough in Yeast

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Abstract

Eukaryotic release factors 1 and 3, encoded by SUP45 and SUP35, respectively, in Saccharomyces cerevisiae, are required for translation termination. Recent studies have shown that, besides these two key factors, several genetic and epigenetic mechanisms modulate the efficiency of translation termination. These mechanisms, through modifying translation termination fidelity, were shown to affect various cellular processes, such as mRNA degradation, and in some cases could confer a beneficial phenotype to the cell. The most studied example of such a mechanism is [PSI+], the prion conformation of Sup35p, which can have pleiotropic effects on growth that vary among different yeast strains. However, genetic loci underlying such readthrough-dependent, background-specific phenotypes have yet to be identified. Here, we used sup35C653R, a partial loss-of-function allele of the SUP35 previously shown to increase readthrough of stop codons and recapitate some [PSI+]-dependent phenotypes, to study the genetic basis of phenotypes revealed by increased translational readthrough in two divergent yeast strains: BY4724 (a laboratory strain) and RM11_1a (a wine strain). We first identified growth conditions in which increased readthrough of stop codons by sup35C653R resulted in different growth responses between these two strains. We then used a recently developed linkage mapping technique, extreme QTL mapping (X-QTL), to identify readthrough-dependent loci for the observed growth differences. We further showed that variation in SKY1, an SR protein kinase, underlies a readthrough-dependent locus observed for growth on diamide and hydrogen peroxide. We found that the allelic state of SKY1 interacts with readthrough level and the genetic background to determine growth rate in these two conditions.

Introduction

High fidelity in translation, one of the key steps in the expression of genetic information, is essential for functional integrity of the cell. Efficient termination is an important aspect of translational fidelity, and a multitude of factors participate in this process [1,2]. The efficiency of translation termination depends on the competition between stop codon recognition by release factors and decoding by near-cognate tRNAs (tRNAs that can pair with two of the three bases of the stop codon) [3]. Recent studies of translation termination in Saccharomyces cerevisiae have revealed genetic and epigenetic regulatory mechanisms that modify translation termination efficiency, which can affect cellular processes such as mRNA degradation and, in some cases, can confer a beneficial phenotype to the cell [4]. The most studied example of such mechanisms is the yeast prion [PSI+], which is formed by a conformational change in Sup35p, a subunit of the translation termination complex [5].

[PSI+] is an epigenetic modifier of translation termination efficiency in S. cerevisiae [6]. Sup35p carries an intrinsically disordered prion-determining region at its amino terminus. When this domain switches to the aggregating amyloid conformation (the prion conformation), much of the protein becomes unavailable for translation terminations, which in turn increases readthrough of stop codons [7,8]. [PSI+] was reported to generate different phenotypes in different genetic backgrounds, and most of these phenotypic effects were shown to be recapitulated by a partial loss-of-function allele of SUP35, sup35C653R [9].

Previous studies have shown that some of the observed [PSI+] dependent phenotypic effects are due to ribosomal frame-shifting [10]. It has also been proposed that some of the observed phenotypic variation in different yeast strains can be due to [PSI+] dependent increase in readthrough, which results in ribosomes bypassing stop codons and reading into regions such as sequences at the 3’ untranslated regions or pseudogenes [11]. These regions are thought to be under less selective pressure than coding sequences, and therefore may be more divergent among different yeast strains. However, specific loci underlying phenotypic differences due to increased readthrough of stop codons have yet to be identified.

Here, we used sup35C653R to examine the phenotypic effects of decreasing translation termination efficiency in various growth conditions in two divergent yeast strains, BY4724 (a laboratory strain hereafter referred to as BY) and RM11_1a (a wine strain...
Author Summary

Proper termination is an important step in a successful mRNA translation event. Many factors, employing genetic and epigenetic mechanisms, are involved in modifying translation termination efficiency in the budding yeast, *Saccharomyces cerevisiae*. [PSI⁺], the prion conformation of Sup35p, one of the translation termination factors in yeast, provides an example of such mechanisms. [PSI⁺] increases readthrough of stop codons. This has the potential to unveil hidden genetic variation that may enhance growth in some yeast strains in certain environments. The specific details of readthrough-dependent phenotypes, however, have remained poorly understood. Here, we used a partial loss-of-function allele of *SUP35*, which increases readthrough of stop codons, and a recently developed linkage mapping technique, X-QTL, to map loci underlying readthrough-dependent growth phenotypes in two divergent yeast strains, BY (a laboratory strain) and RM (a wine strain). We found that readthrough-dependent growth phenotypes are often complex, with multiple loci influencing growth. We also showed that variants in the gene *SKY1* underlie one of the loci detected for readthrough-dependent growth phenotypes in the presence of two chemicals that induce oxidative stress.

X-QTL reveals readthrough-dependent loci

We used X-QTL [14] to examine the genetic basis of the observed readthrough-dependent differences in growth rate ratio between BY and RM. For each growth condition, we performed X-QTL on two segregant pools in parallel: a wildtype pool from a cross between wildtype BY and RM, and a sup35 pool from a cross between BY and RM both carrying sup35 and [psi⁺] (Materials and Methods). We grew these pools on selection plates (rich medium plus the chemical agent of interest) and control plates (Materials and Methods), and compared the allele frequencies between the selected pools and control pools by microarray-based single nucleotide polymorphism (SNP) genotyping as previously described [14]. A locus that affects growth rate in a given condition independent of sup35 is expected to be detected as an allele frequency skew of similar direction and magnitude in both the wildtype and sup35 selected pools. In contrast, a locus whose effects depend on sup35 is expected to show a difference in the allele frequency skew between the two pools. The number of loci detected for growth rates in the nine conditions at an FDR of 5% ranged from one to 20 in both wildtype and sup35 crosses (Figure S2A–S2I). The results in the wildtype cross were similar to those previously described for these growth conditions [14], which showed the reproducibility of X-QTL. Most loci showed similar allele frequency skews in the wildtype and sup35 pools; however, 18 loci showed significant differences between these pools (FDR = 5%; Materials and Methods) (Figure S2A–S2I). We refer to these loci as “readthrough-dependent”. One to six readthrough-dependent loci were detected in the growth conditions tested (Figure 2A). These results showed that sup35-mediated effects on growth in certain conditions are genetically complex, as was previously suggested for some [PSI⁺]-dependent growth phenotypes [9]. Each readthrough-
Figure 1. Readthrough-dependent strain-specific growth effects. The ratio of sup35 strain growth rate and wildtype strain growth rate is plotted (mean ± SD) for BY (orange bars) and RM (purple bars) for the nine conditions in which the effect of sup35<sup>C653R</sup>-mediated increase in readthrough on growth differed between the two strain backgrounds (uncorrected \( p < 0.05 \), FDR = 10%).

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Figure 2. Readthrough-dependent loci. A) Loci detected for each growth condition as significantly different between X-QTL allele frequencies in wildtype and sup35 selected pools (FDR 5%, Materials and Methods). Readthrough-dependent loci that were also called significant by an alternative statistical approach are marked with *. B) Histogram of the number of growth conditions for each readthrough-dependent locus.

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dependent loci had an effect in one to five conditions, with a total of ten distinct loci detected (Figure 2B).

**SKY1** variation underlies the readthrough-dependent locus on chromosome XIII

In order to gain more insight into readthrough-dependent effects on growth rate, we focused on the loci on the right arm of chromosome XIII, which affected growth in five conditions (Figure 2B). We chose one of the growth conditions in which this locus had the strongest effect for further investigation: diamide, a sulfhydryl-oxidizing agent [18] (Figure S2D). At this locus, we detected a frequency skew in favor of the RM allele in the sup35 pool but not in the wildtype pool, which suggested that in the presence of increased readthrough, strains carrying an RM allele at this locus grow better on diamide than strains carrying the BY allele.

Based on sequence comparison between BY and RM for the genes in this region (Figure S3), we selected **SKY1** and **MRE11** for further investigation, as both contained nonsynonymous changes in their open reading frames and in the downstream regions that might be translated due to increased readthrough. Comparison of the coding sequence of **SKY1** between BY and RM showed 13 single nucleotide polymorphisms (SNPs) between the two strains, including seven nonsynonymous substitutions. The downstream sequence contained a two-nucleotide deletion in BY at the 129th nucleotide after stop codon, which results in the addition of three amino acids in BY before the next stop codon is reached. Comparison of the coding sequence of **MRE11** between BY and RM showed eight SNPs between the two strains, including five nonsynonymous substitutions. The downstream sequence contained five SNPs, including four nonsynonymous substitutions.

To test the causality of **SKY1** and **MRE11** polymorphisms for the effects of this locus, we replaced the **SKY1** and **MRE11** genes in both wildtype and sup35 RM with the BY versions. For both genes, we replaced the downstream sequence along with the coding sequence. We previously showed that the expression level of **SKY1** is lower in RM than in BY, and that this difference maps to the location of the **SKY1** gene, suggesting the presence of a cis-regulatory polymorphism [19]. Therefore, we included the upstream regulatory region in the **SKY1** allele replacement along with the coding and downstream sequence. We then repeated the X-QTL experiments for growth on diamide with both wildtype and sup35 segregant pools from crosses using the RM parent strains with **SKY1** and **MRE11** allele replacements (that is, both parents carried the BY allele of **SKY1** or **MRE11**, respectively). In the crosses with the **MRE11** allele replacement, the results were unchanged; that is, we still saw a skew in the direction of the RM allele at this locus in the sup35 pool despite the fact that **MRE11** was no longer polymorphic, ruling it out as the causal gene for this locus (Figure 3A). In contrast, this allele frequency skew disappeared in the sup35 pool from the cross with the **SKY1** allele replacement, and there was no longer any difference in allele frequency at this locus between the wildtype and sup35 pools (Figure 3B). These results demonstrate that polymorphisms in **SKY1** are causal for the effects of this locus, and that the difference in growth between the RM and BY alleles of **SKY1** is revealed when readthrough is increased from the wildtype level by sup35.**

To better understand the effect of **SKY1** on the readthrough-dependent difference in growth rate between BY and RM on diamide, we measured growth rates of **SKY1**-swapped wildtype and sup35 BY and RM and compared them to the original strains. For this experiment, we constructed wildtype and sup35 BY strains carrying the RM allele of **SKY1**. Similar to the previous replacements strains, we replaced the upstream regulatory region along with the coding and downstream sequences of **SKY1**. We also measured growth rates of wildtype and sup35 BY and RM strains with **SKY1** knocked out (sky1A strains). In the presence of sky1A, there was no significant difference in growth between wildtype and sup35 strains in either genetic background (Figure 4A), implying that readthrough-dependent differences in growth are mediated by **SKY1**. As expected from the X-QTL results, replacing **SKY1** in the BY background with the RM allele increased growth rate in diamide in the presence of sup35, and replacing **SKY1** in the RM background with the BY allele decreased growth rate in the presence of sup35 (Figure 4B). These results confirmed the growth effects of **SKY1** polymorphisms.

A readthrough-dependent locus for growth on hydrogen peroxide was also observed at this genomic location (Figure 2B, Figure S2G). Therefore, we tested whether **SKY1** polymorphisms underlie the effects of this locus on hydrogen peroxide as well. X-QTL experiments with allele replacement strains showed that polymorphisms in **SKY1** are indeed causal (Figure S4). Growth rate measurements in wildtype and sup35 BY and RM strains and the corresponding **SKY1** swapped and sky1A strains confirmed the effects of **SKY1** polymorphisms on growth in the presence of hydrogen peroxide (Figure S5A–S5B).

**Complex interplay between sup35 effects, allelic state of SKY1,** and genetic background determines growth rate on diamide and hydrogen peroxide

When we first measured the growth rates of wildtype and sup35 BY and RM in the presence of diamide, we found that BY-sup35 grew significantly slower than wildtype BY, while there was little difference in growth rate between RM-sup35 and wildtype RM (Figure 4C). When the BY allele of **SKY1** was replaced with the RM allele, the difference in growth between the sup35 and wildtype strains was reduced, although it remained significant (Figure 4C). When the RM allele of **SKY1** was replaced with the BY allele, the sup35 strain grew somewhat slower than the wildtype strain (Figure 4C). We found similar results for growth of these strains in presence of hydrogen peroxide (Figure S5C). The observation that **SKY1** replacement strains recapitulated the direction but not the magnitude of the effects of readthrough on growth in these conditions seen in the parent strains suggested the presence of interactions between **SKY1** polymorphisms, readthrough, and genetic background. We used Analysis of Variance (ANOVA) (Materials and Methods) to formally test the effects of these factors and the interactions among them (Table 1, Table S2). The model showed a major effect of the genetic background, with RM growing better in presence of diamide and hydrogen peroxide than BY. **SKY1** allelic status also had a significant effect, with the RM allele increasing growth. Readthrough level did not show a significant effect on its own but did show significant interaction effects with both genetic background and **SKY1** allelic status.

These results suggest a complex interplay between the effects of sup35-mediated increase in readthrough, the allelic state of **SKY1**, and genetic background in determining growth on diamide and hydrogen peroxide. At wildtype readthrough levels, RM grows better than BY, and swapping **SKY1** in either strain with the version from the other strain has little effect (Figure 4D, Figure S5D). When readthrough is increased by introduction of sup35, growth rate decreases dramatically in BY, but shows no change in RM (Figure 4D, Figure S5D). The slower growth rate of BY-sup35 in comparison to wildtype BY is partially rescued by introduction of the RM allele of **SKY1** and completely rescued by knocking out **SKY1** (Figure 4D, Figure S5D). Introduction of the BY allele of **SKY1** into RM-sup35 reduces the growth rate of
this strain but not to the extent seen in the BY background (Figure 4D, Figure S5D). Thus, \textit{sup35C653R} and the BY allele of \textit{SKY1} act together to lower growth rate on diamide and hydrogen peroxide, and this effect is accentuated by as yet unidentified factors in the BY genetic background.

Given the evidence for \textit{cis}-regulatory polymorphism in \textit{SKY1} that lowers expression of the RM allele, we investigated whether differences in transcript abundance could account for the allelic effect of \textit{SKY1}. We used quantitative RT-PCR to measure \textit{SKY1} mRNA levels in wildtype and \textit{sup35} BY and RM, as well as in the corresponding \textit{SKY1}-replaced strains (Materials and Methods). We found that \textit{SKY1} mRNA levels were independent of the growth condition used (Figure 5). As expected based on microarray data [19] we found that \textit{SKY1} expression is higher in wildtype BY than in wildtype RM. Moreover, we found that swapping \textit{SKY1} in wildtype BY and wildtype RM with the alternate allele changed \textit{SKY1} expression level to the alternative level. These results confirm the presence of \textit{cis}-regulatory polymorphism that alters the expression level of \textit{SKY1} between BY and RM. Surprisingly, in the RM background, increasing readthrough from the wildtype level to the \textit{sup35} level resulted in roughly a ten-fold drop in \textit{SKY1} expression level, while no change was observed in BY-\textit{sup35}. This drop in \textit{SKY1} mRNA in RM-\textit{sup35} was largely reversed by swapping in the BY allele of \textit{SKY1} (Figure 5). We used ANOVA to model the effect of the measured \textit{SKY1} mRNA abundance on growth rate, and then used the residual growth rate to test whether the allelic effect of \textit{SKY1} was changed. The results suggested that the readthrough-dependent growth effects of \textit{SKY1} are not mediated by mRNA levels (Table 2, Table S3).

To gain further mechanistic insight into how \textit{sup35C653R} leads to the differential allelic effects of \textit{SKY1}, we swapped just the downstream sequences of \textit{SKY1} in both wildtype and \textit{sup35} BY and RM with the alternative alleles. These replacement strains differ from the parent strains only by the polymorphism that extends the C-terminus. Swapping this polymorphism alone captured the allelic effect of \textit{SKY1} in both growth conditions (Figure 6, Figure S6), consistent with a differential effect of readthrough on the downstream regions from the two strains. To test whether the allelic effect of \textit{SKY1} is directly related to translational readthrough, we then introduced a second stop codon immediately

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**Figure 3. \textit{SKY1} variation underlies the locus on Chromosome XIII for growth on diamide.** A) Comparisons of the selected segregating population (Selection) to the whole population (Control) from a cross between \textit{sup35} BY and RM parent strains (\textit{sup35}) and a cross between wildtype parent strains (WT) are shown for \textit{MRE11}-fixed populations (BY x RM- \textit{MRE11}BY) in presence of diamide. For plotting, average of two biological replicates is used for each selection and control. Sliding window averages (40 kb) are plotted. Enrichment of the BY allele is indicated by deviations above zero and enrichment of the RM allele is indicated by deviations below zero. B) Comparisons of the selected segregating population (Selection) to the whole population (Control) from a cross between \textit{sup35} BY and RM parent strains (\textit{sup35}) and a cross between wildtype parent strains (WT) are shown for \textit{SKY1}-fixed populations (BY x RM- \textit{SKY1}BY) in presence of diamide. Average of two biological replicates is used for each selection and control. The dotted line shows the interval surrounding \textit{MRE11} and \textit{SKY1}.

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after the native stop codon in wildtype and sup35 BY and RM strains. Introducing a second stop codon in wildtype BY and RM did not affect growth rates of these strains in diamide or hydrogen peroxide (Figure 6, Figure S6). In the presence of the sup35 mutation, the growth rate in the BY strain with the second stop codon rose to the same level as when the SKY1 allele is replaced with the RM version, while the second stop codon did not alter growth rate in RM (Figure 6, Figure S6). These results support the hypothesis that increased readthrough of the BY downstream region due to the sup35 mutation causes reduced growth in diamide and hydrogen peroxide, perhaps because translation of this region stabilizes Sky1p.

Discussion

Modifying translational readthrough in S. cerevisiae has been shown to affect yeast cells in various ways [4]. The prion [PSI+] provides one example of translational readthrough modification in yeast cells. Previous works have shown that [PSI+] can reveal hidden phenotypic variation among yeast strains, that this effect is largely
recapitulated by the \(\text{sup}^{\text{C653R}}\) mutation, which increases translational readthrough, and that the resulting phenotypic differences may have a complex genetic basis \([9,16]\). Here, we have advanced our understanding of the genetic basis of readthrough-dependent phenotypes by identifying specific loci that underlie hidden variation revealed by \(\text{sup}^{\text{C653R}}\). Using this partial-loss-of-function allele of \(\text{SUP35}\) allowed us to focus on distinct hidden phenotypes in \(\text{BY}\) and \(\text{RM}\) revealed by increased translational readthrough. Our growth rate measurements in diverse stressful conditions for wildtype (WT) and \(\text{sup35}\) \(\text{BY}\) and \(\text{RM}\) showed that \(\text{sup35}\)-mediated differences in growth between these two strains were relatively modest compared to previous studies of \([\text{PSI}^+]\)-mediated effects \([9,16]\). This could potentially be explained by other \([\text{PSI}^+]\)-dependent phenotypic effects in yeast, such as ribosomal frame shifting \([10]\) or the presence of \(\text{Sup35}\) prion aggregates \([20]\), which are absent in our system. We consider it more likely, given the reported recapitulation of most \([\text{PSI}^+]\) strain-dependent phenotypic effects with the \(\text{sup}^{\text{C653R}}\) mutation \([9]\), that this difference in effect sizes is due to the different genetic backgrounds used.

We found that \(\text{sup35}\)-mediated increase in readthrough had different effects on growth rates in \(\text{BY}\) and \(\text{RM}\) for about one-quarter of the growth conditions tested. Our mapping results lend additional support to the previously reported inference that some readthrough-dependent growth phenotypes are genetically complex based on their segregation patterns \([9]\), and further suggest that some of the underlying loci have small effect sizes.

We showed that \(\text{SKY1}\) is the causal gene underlying the strongest readthrough-dependent locus detected for growth in the

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**Table 2.** Modeling growth rate in the presence of diamide after regressing out \(\text{SKY1}\) mRNA data using ANOVA with three factors: genetic background (BG), \(\text{SUP35}\) allelic status, and \(\text{SKY1}\) allelic status.

| Coefficients | Estimate | Std. Error | t value | Pr (>|t|) |
|--------------|----------|------------|---------|-----------|
| (Intercept)  | 0.0802   | 0.00973    | 8.19    | 3.7E-11   |
| BG (BY)      | -0.160   | 0.0138     | -11.57  | <2e-16    |
| SUP35 (WT)   | -0.00604 | 0.0138     | -0.436  | 0.665     |
| SKY1 (BY)    | -0.08668 | 0.0138     | -6.27   | 5.50E-08  |
| BG (BY): SUP35 (WT) | 0.0737 | 0.0196 | 3.76 | 4.10E-04  |
| BG (BY): SKY1 (BY) | 0.0299 | 0.0196 | 1.53 | 0.133     |
| SUP35 (WT): SKY1 (BY) | 0.101  | 0.0196 | 5.14 | 3.67E-06  |
| BG (BY): SUP35 (WT): SKY1 (BY) | -0.038 | 0.0277 | -1.37 | 0.176     |

**Figure 5.** Measured \(\text{SKY1}\) mRNA levels in YPD, diamide, and hydrogen peroxide. \(\text{SKY1}\) mRNA level measured via quantitative RT-PCR is compared for wildtype (WT) and \(\text{sup35}\) \(\text{BY}\) and \(\text{RM}\) as well as \(\text{BY-SKY1}^{\text{RM}}\) and \(\text{RM-SKY1}^{\text{BY}}\) in rich media (A), rich media plus diamide (B) and rich media plus hydrogen peroxide (C). Measurements are shown relative to the mRNA levels in wildtype \(\text{BY}\) in YPD. \(\text{SKY1}\) replacement strains were made by swapping the \(\text{SKY1}\) upstream regulatory region, open reading frame, and downstream sequence.

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presence of diamide and hydrogen peroxide. Our results suggest that translation of the BY downstream sequence of SKY1 is disadvantageous for growth in these conditions. We found that a complex interplay between the genetic background, SKY1 allelic state, and sup35 determines growth rate in these two conditions. SKY1 mRNA measurements showed that the readthrough-dependent effect of SKY1 on the growth differences between BY and RM is not mediated by mRNA levels. However, we observed a dramatic drop in the SKY1 mRNA level in sup35 RM relative to wildtype RM, while we did not see a drop in the SKY1 mRNA level in sup35 BY relative to wildtype BY. One mechanism that could explain the sup35-mediated drop in the SKY1 mRNA level in RM is Nonstop mRNA Decay (NSD) pathway [21], which might be differentially active in BY and RM. This mRNA surveillance mechanism is initiated when the ribosome reaches the 3′ end of the mRNA, and therefore eliminates transcripts lacking stop codons or transcripts that have stop codons that were bypassed during translation. Ribosomes are more likely to reach the 3′ end of an mRNA after reading through one or more stop codons in sup35 strains than in wildtype strains. Therefore, NSD is also more likely to eliminate such mRNAs in the presence of sup35. Importantly, even a single ribosome that reaches the 3′ end of an mRNA is predicted to trigger NSD, resulting in the reduction of the mRNA level [22]. The decrease in SKY1 mRNA caused by sup35 in the RM background is largely rescued by swapping in the BY allele of sup35, which suggests that the combination of increased readthrough and NSD can act in an allele-specific fashion.

Sky1p is a protein kinase that phosphorylates SR proteins [23], proteins with domains containing alternating serine and arginine residues which are components of the machinery for the processing [24] and nuclear export [25] of mRNAs. One of the known Sky1p targets, Npl3p, was shown to promote translation termination accuracy in yeast [26]. However, the same paper showed that the role of Npl3p in translation termination is independent of the posttranslational modifications mediated by Sky1p. Sky1p has also been shown to regulate cation homeostasis and salt tolerance [27]. Deletion of SKY1 confers resistance to several anticancer drugs, such as cisplatin and carboplatin [28], and to polyamine toxic analogues [29]. Several hypotheses have been proposed to explain the role of Sky1p in resistance to these drugs, such as a Sky1p-mediated effect on splicing or transport of target mRNAs, regulation of membrane permeability, and regulation of drug uptake. However, the direct target(s) of Sky1p that mediate these effects are unknown. Our results demonstrate that deletion of SKY1 also confers a growth advantage in the presence of oxidative stress inducers diamide and hydrogen peroxide. A genetic interaction between SKY1 and SUP35 was previously reported in the S288c background in rich media [30]. Here we showed that a genetic interaction is present between SKY1 and SUP35 in the presence of diamide and hydrogen peroxide in the BY background but not in the RM background. These results support previous finding that readthrough-dependent phenotypes vary based on the genetic background [9].

Materials and Methods

Strains, media, and plasmids

Cultures were grown in minimal medium containing 0.67% (w/v) yeast nitrogen base without amino acids (Difco) containing 2% (w/v) glucose (SMD) or rich medium, as specified. Additional nutritional supplements or drugs were added as required. YPD plates were made as described [31]. For sporulation, SPO++ was used (http://www.genomics.princeton.edu/dunham/sporulationdissection.htm).

We used pEF675 (a kind gift from Eric Foss) to replace SUP35 with sup35C653R via two-step allele replacement [32] in BY4724 (Mat\(\alpha\) ura3Δ lys2Δ) [33] and RM11-1a (Mat\(\alpha\) ura3Δ his3Δ0::NatMX hox::HphMX AMV1\(^{pp}\)), pEF675 was made by sub-cloning Sup35 into a common URA3-marked integrating yeast plasmid (pRS306 [34]) and subsequently changing cysteine 563 (TGT) to an arginine (CGT). Successful replacement for each strain was then confirmed by sequencing. We refer to strains with SUP35 as wildtype and strains with sup33C653R as sup35. We then transferred sup35 into strains with suitable genetic markers for X-QTL. To do so, we crossed BY-sup33 and RM-sup35 strains into BY MAT\(\alpha\) can1Δ::STE2pr-SpHIS5 hyp1Δ his3Δ1 and RM MAT\(\alpha\) AMV1\(^{pp}\) his3Δ0::NatMX hox::HphMX [14], respectively. After sporulating the obtained diploids and genotyping the dissected tetrads, we selected BY MAT\(\alpha\) his3Δ1 hyp1Δ can1Δ::STE2pr-SpHIS5 sup35C653R.
and RM Matα AMY1ΔAT his3Δ0::NATMX hOΔ::HphMX sup35ΔC653R as sup35 parental strains in X-QTL. We used strains form [14] as wildtype parental strains in X-QTL. 

SKT1 and MRE11 replacement strains were generated by a two-step replacement method [35]. Each gene was first replaced with \( URA3^+ \) cassette from pCORE in wildtype and \( sup35^+ \) BY and RM strains generating goa::URA3-KanMX knockout strains. SKT1 and MRE11 alleles from the donor strains were amplified by PCR and introduced into recipient strains to replace \( URA3^+ \) cassette. Where mentioned, 400-base pair from the upstream regulatory region or 300-base pair from the downstream region is included in making replacement strains. Allele replacement were confirmed by sequencing.

### Sequencing

The RM SKT1 and MRE11 sequences were obtained from the whole genome-sequencing project at the Broad Institute (http://www.broad.mit.edu/annotation/genome/saccharomyces_cerevisiae/). All sequencing was done using standard dideoxy methods.

### Growth rate measurement

We inoculated strains under examination in a 96-well plate (Costar; 3370) in rich medium and incubated the plate in 30°C until saturation. We then used a sterile 96-pin replicator (Nunc; 62409-606) to inoculate Costar 96-well plate (3370) containing rich medium and the reagent of interest. We then used Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments) set at the desired temperature (30°C unless mentioned otherwise) and with continuous shaking at medium speed to collect \( \mathrm{OD}_{600} \) at 30-minute intervals for up to 20 hours. We used data points corresponding to 0.05<\( \mathrm{OD}_{600} \)<0.5 (logarithmic growth phase) to calculate growth rate. Growth rate was calculated as the slope of a linear regression of the log transformed logarithmic growth phase data points. For each strain, unless otherwise specified, growth rate in rich media plus the reagent of interest is normalized by the strain’s growth rate in rich media. Growth rate is shown as the mean \( \pm \) standard deviation of values obtained from at least eight independent growth measurements, including at least four biological replicates. We then performed t-test comparison between BY and RM growth rate ratios for all growth conditions. We then performed t-test comparison of eight independent growth measurements, including at least four biological replicates. We then performed t-test comparison of eight independent growth measurements, including at least four biological replicates.

### Dual luciferase assay

Dual luciferase assay was performed as explained before [12]. Plasmids with the stop codon (pDB691) or the sense codon (pDB690), kindly provided by David Bedwell (University of Alabama at Birmingham), were transformed into the indicated yeast strains, and transformants were selected on SMD-drop out plates lacking uracil. Transformed strains were grown in liquid SMD medium to a cell density of 0.5–0.7 \( A_600 \) units/mL, as measured using Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments). The luciferase assay was performed using the Dual-Luciferase Reporter Assay System (Promega; E1910). Approximately 10\(^4\) yeast cells from each strain expressing the indicated dual luciferase reporter were lysed using 100 \( \mu \)L of Passive Lysis Buffer in a 96-well plate (Costar; 3370). Two microliters of the lysate were added to 10 \( \mu \)L of the Luciferase Assay Reagent II in an opaque 96-well plate (Costar; 3614). Relative luminescence units (RLUs) produced by firefly luciferase activity were then measured for 10 seconds using Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments). 10 \( \mu \)L of Stop&Glo buffer was then added to quench the firefly activity and activate the Renilla luciferase activity. RLU's were again measured for 10 seconds to determine the Renilla luciferase activity. Negative controls that contained all the reaction components except cell lysates were used to determine the background for each luciferase reaction and were subtracted from the experimental values obtained. Percent readthrough is expressed as the mean \( \pm \) the standard deviation of values obtained from at least eight independent dual luciferase assay including at least four biological replicates.

### X-QTL

For each growth condition, we performed X-QTL on two biological replicates for the wildtype BY×RM cross and two biological replicates for the \( sup35^+ \) cross. Matα haploid segregants from the indicated cross were selected as explained before [14]. To create the segregating pool, a single colony of the diploid progenitor of the mentioned cross was inoculated into 5 mL YPD and grown to stationary phase. The diploid culture was spun down and the supernatant was decanted. The diploid pellets were then resuspended in 50 mL SPO++ sporation medium. The sporation was kept at room temperature (\( \sim 22^\circ \)C) with shaking and monitored for the fraction of diploids that had spordulated. Once more than 50% of the diploids had spordulated, 10 mL of the sporation were spun down and then the supernatant was decanted. The pellet was resuspended in 2 mL water. 600 \( \mu \)L \( \beta \)-glucoronidase (Sigma; G7770) were added to the preparation, and the mixture was incubated at 30°C for one hour. Water was added to the sample to the total volume of 20 mL. The spore preparation was spread onto SMD+canavamine/thiavlyine plates (Sigma; C9758 for canavamine-L-canavamine sulphate salt; A2636 for thiavlyine (S-(2-aminoethyl)-L-cysteine hydrochloride)), with 100 \( \mu \)L of sample going onto each plate. The plates were incubated at 30°C for two days. Then 10 mL of water were poured onto each plate and a sterile spreader was used to remove the segregants from the plate. The cell mixtures from four plates were then pipettet off the plates into a container. The pool was spun down and the water decanted. Haploid segregants were then inoculated into 100 mL liquid YPD and were incubated in 30°C while shaking on a rotary shaker at 200 rpm for about 30 minutes to recover. After the recovery, 100 \( \mu \)L of the segreagant pool was pipetted and spread on the selection plates (YPD+reagent of interest) and control plates (YPD). For each condition/cross we used five selection plates to pool the resistant segregants. For each cross, we used three control plates to pool the whole population of segregants. Selection and control plates were then incubated at 30°C for two days. DNA was extracted from the grown cells using Genomic-tip 100/G columns (Qiagen; 10243). DNA was labeled using the BioPrime Array CGH Genomic Labeling Module (Invitrogen; 18095-012) with the sample being labeled with Cy3 dUTP and the reference being labeled with Cy5 dUTP. We used a BY/RM diploid as the reference for all hybridizations. Labeled samples were then hybridized onto the allele-specific genotyping microarray with isothermal probes that assay single nucleotide polymorphisms (SNPs) between BY and RM [14]. The array data have been deposited in NCBI’s Gene Expression Omnibus (GEO) [37] and are accessible through GEO Series accession number GSE33817 [http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33817]. Hybridization intensities were extracted and normalized using the rank invariant method in the Agilent Feature Extraction software package. For a given SNP, the difference in the log_{10} ratios of BY and RM-specific probes on a single array (or log_{10} intensity difference) was computed. Background allele frequency changes that occur during
pool construction were removed from the selection by subtracting the log10 intensity difference obtained for the whole (control) population from the log10 intensity difference observed in the selections. To find readthrough-dependent peaks, we used a Savitzky-Golay filter to smooth the input data within sliding windows of 100 probes. The Savitzky-Golay method essentially performs a local polynomial regression on a series of values to determine the smoothed value for each point. This smoothing approach was used to preserve local maxima in the data. For each probe, we subtracted the average of the two wildtype X-QTL replicates from the average of the two sup35 X-QTL replicates for each growth condition and used this measure as the input data. Readthrough-dependent loci were called if the smoothed value surpassed the threshold for a 5% FDR, where the number of false discoveries at each threshold was determined by using the same algorithm on the control data, which were obtained by subtracting wildtype control X-QTL results from sup35 control X-QTL results for growth on YPD. To ensure that the results are robust to the statistical approach used, we also performed a student’s t-test comparison of a moving window of six probes from the background-subtracted data for the two wildtype and the two mutant replicates for each selection. We set the threshold at \( p \text{ value} = 2.78 \times 10^{-6} \) (Bonferroni-corrected \( p < 0.05 \)). Peak calling and all other statistical analyses were conducted in R (http://www.r-project.org/).

**ANOVA**

For growth rate in diamide and hydrogen peroxide, an ANOVA of the form

\[
\text{Growth rate} \sim \text{BG} \times \text{sup35} \times \text{SKY1}
\]

was performed in R using the \( \text{lm} \) function. BG stands for genetic background, which can be either BY or RM. \( \text{sup35} \) stands for the allelic status of \( \text{SUP35} \), which can be either wildtype or \( \text{sup35}C653R \). \( \text{SKY1} \) stands for the allelic status of \( \text{SKY1} \), which can be either BY allele or RM allele. To test whether \( \text{SKY1} \) mRNA level could account for \( \text{SKY1} \) allelic effect, we first used an ANOVA of the form

\[
\text{Growth rate} \sim \text{mRNA}
\]

where mRNA stand for \( \text{SKY1} \) mRNA level and then used the residuals in an ANOVA of the form mentioned above.

**Quantitative RT–PCR**

Each quantitative RT-PCR measurement represents data collected from three biological replicates. We harvested cells from the logarithmically growing strains in the mentioned growth conditions. We then used total RNA extraction kit (Norgen; 17200) to extract total RNA from collected cells. To perform quantitative RT-PCR, we used TaqMan RNA-to-C\(_T\) one-step kit (Applied Biosystems; 4393463G) and 7900HT Fast Real-Time PCR System (Applied Biosystems; 4329001). We used TaqMan TAMRA probes. We used a 6-FAM labeled probe for \( \text{SKY1} \) detection and a VIC labeled probe for \( \text{TDH2} \) detection (internal control). The primers were selected so that there would be no polymorphisms in the sequence amplified for \( \text{SKY1} \) (185-base pair fragment starting at 403\(^{\text{th}}\) nucleotide of \( \text{SKY1} \) coding sequence) and \( \text{TDH2} \) (168-base pair fragment starting at 379\(^{\text{th}}\) nucleotide of \( \text{TDH2} \) coding sequence). The sequences of primers and probes used are as follows:

\[
\text{SKY1-F:} \quad \text{ACTAAATGAGCGTGACFCTTT} \\
\text{SKY1-probe:} \quad \text{TCTTTGAAGATTACAGGCAGGTG} \\
\text{TDH2-F:} \quad \text{AGGTTGTCATCCTGCTCCAT} \\
\text{TDH2-R:} \quad \text{GTGGTGAAAGGCGTTGGA} \\
\text{TDH2-probe:} \quad \text{CCAATGTGTCGTATGGGTTAACG}
\]

**Supporting Information**

**Figure S1** Partial loss of function allele of \( \text{SUP35} \) (\( \text{sup35}C653R \)) increases readthrough in BY and RM. Replacing wildtype allele of \( \text{SUP35} \) with the partial loss of function allele (\( \text{sup35}C653R \)) increases %readthrough in both BY and RM. %Readthrough was measured via a dual luciferase reporter assay, which uses tandem \( \text{Renilla} \) and firefly luciferase genes that are separated by a single in-frame stop codon. The activity of the firefly luciferase, encoded by the distal open reading frame, provides a quantitative measure of the readthrough of the stop codon that separates the two open reading frames. The activity of the \( \text{Renilla} \) luciferase, encoded by the proximal open reading frame, serves as an internal control for mRNA abundance. Thus, the relative abundance of these light-emitting proteins measures the efficiency of translation termination. Here, we used two separate reporters; one with UGA (stop codon) and one with CGA (sense codon) separating the \( \text{Renilla} \) and firefly open reading frames. For each strain, we calculated the readthrough as the ratio of firefly to \( \text{Renilla} \) luciferase activity in the presence of the stop codon, normalized by the observed ratio for the sense codon constructs.

(TIF)

**Figure S2** A. X-QTL results for growth on chlorpromazine. Result for segregrants from a cross between \( \text{sup35} \) BY and RM, wildtype BY and RM, and the t-test comparison between wildtype and \( \text{sup35} \) results is shown. The top two plots show comparisons of the allele frequencies from selected segregating population (Selection) to the whole population (Control) from a cross between \( \text{sup35} \) parent strains (\( \text{sup35} \)) and from a cross between wildtype parent strains (WT). For plotting, average of two biological replicates is used for each selection and average of six biological replicates is used for each control. Sliding window averages (40 kb) are plotted. Enrichment of the BY allele is indicated by deviations above zero and enrichment of the RM allele is indicated by deviations below zero. The third plot shows the readthrough-dependent loci (marked with dotted lines) called using an smoothing algorithm based on Savitzky-Golay filter on the differences between allele-frequency skews for wildtype and \( \text{sup35} \) X-QTL results (FDR 5%, Materials and Methods). The bottom plot shows \( -\log(10) \) obtained from t-test comparison between allele frequencies in wildtype and \( \text{sup35} \) selected pools. When present, the readthrough-dependent loci \( (p < 2.78 \times 10^{-6}; \text{Bonferroni-corrected} \ p < 0.05) \) are marked with dotted lines. Results are represented in the same manner for (B–I). B. X-QTL results for growth on cobalt chloride. C. X-QTL results for growth on cycloheximide. D. X-QTL results for growth on diamide. E. X-QTL results for growth on E6-berbamine. F. X-QTL results for growth on ethanol. G. X-QTL results for growth on hydrogen peroxide. H. X-QTL results for growth on neomycin. I. X-QTL results for growth on tunicamycin.

(TIFF)

**Figure S3** Interval corresponding to readthrough-dependent locus detected for growth in presence of diamide on Chromosome XIII. 50 kb surrounding the region on chromosome XIII and the genes residing in the region is shown (http://www.yeastgenome.org/).

(TIF)
Figure S4  SKY1 variation underlies the locus on Chromosome XIII for growth on hydrogen peroxide. A) Comparisons of the selected segregating population (Selection) to the whole population (Control) from a cross between sup35 BY and RM parent strains (sup35) and a cross between wildtype parent strains (WT) are shown for MRE11-fixed populations (BY×RM- MRE11BY) in hydrogen peroxide. For plotting, average of two biological replicates is used for each selection and control. B) Comparisons of the selected segregating population (Selection) to the whole population (Control) from a cross between sup35 BY and RM parent strains (sup35) and a cross between wildtype parent strains (WT) are shown for SKY1-fixed populations (BY×RM- SKY1BY) in hydrogen peroxide. For plotting, average of two biological replicates is used for each selection and control. The dotted line shows the interval surrounding MRE11 and SKY1.

(TIF)

Figure S5  SKY1 variation contributes to readthrough-dependent differences in growth rates of BY and RM on H₂O₂. A) Knocking out SKY1 eliminates the observed difference between wildtype and sup35 growth rates in BY background. B) Replacing SKY1 (upstream regulatory region, open reading frame and the downstream sequence) in the BY background with the RM allele increased growth rate in diamide in the presence of sup35, and replacing SKY1 (upstream regulatory region, open reading frame and the downstream sequence) in the RM background with the BY allele decreased growth rate in the presence of sup35. C and D) these panels show the data presented in panels A and B grouped in different ways to highlight the growth effects of the sup35 allelic state and the growth effects of the genetic background. Growth rates of wildtype and sup35 BY and RM, as well as the corresponding SKY1 swapped and sky1A strains are shown for growth in the presence of hydrogen peroxide, grouped according to the sup35 allelic state (C) and genetic background (D). For each strain, growth rates are normalized based on the strain’s growth rate in rich medium (YPD).

(TIF)

Figure S6  Readthrough-dependent strain-specific growth effects of SKY1 in hydrogen peroxide. Replacing the SKY1 downstream sequence alone captures the allelic effects of SKY1 in sup33 strains. Introducing a second stop codon immediately after the native stop codon at the end of the SKY1 open reading frame shows that strain-specific growth effects of SKY1 polymorphism are readthrough-dependent.

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Author Contributions

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