

Genetic interactions between $[PSI^+]$ and nonstop mRNA decay affect phenotypic variation

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Yeast strains can reversibly interconvert between $[PSI^+]$ and $[psi^-]$ states. The $[PSI^+]$ state is caused by a prion form of the translation termination factor eRF3. The $[PSI^+]$ state causes read-through at stop codons and can lead to phenotypic variation, although the molecular mechanisms causing those phenotypic changes remain unknown. We identify an interaction between $[PSI^+]$ -induced phenotypic variation and defects in nonstop mRNA decay. Nonstop mRNA decay is triggered when a ribosome reaches the 3' end of the transcript. In contrast, we observed little interaction between $[PSI^+]$ -induced phenotypic variation and defects in nonsense-mediated decay, which lead to suppression of premature stop codons. These results suggest that at least some of the phenotypic effects of $[PSI^+]$ may be due to read-through of "normal" stop codons, thereby producing extended proteins. Moreover, these observations suggest that nonstop mRNA decay may limit $[PSI^+]$ -induced phenotypic variation. Such a process would allow periodic sampling of the 3' UTR, which can diverge rapidly, for novel and beneficial protein extensions.

capacitor | evolution | prion

The $[PSI^+]$ state is a heritable characteristic of yeast that shares key characteristics with prions (reviewed in refs. 1–4). eRF3 is one of two key factors that function in the termination of translation at stop codons (5, 6). In $[psi^-]$ cells the eRF3 protein is in a conformation that is functional in translation termination, which occurs efficiently. In contrast, in $[PSI^+]$ cells the majority of eRF3 is in an altered conformation, and $[PSI^+]$ cells have a reduced efficiency of translation termination (3, 4). Both the $[PSI^+]$ and $[psi^-]$ states are relatively stable, because the preexisting prion form of eRF3 is needed to efficiently convert eRF3 to the prion form. However, the stability is not absolute, and $[PSI^+]$ cells convert to $[psi^-]$ and vice versa at a low rate (1, 2).

The functions of the eRF3 protein in translation termination and prion propagation are easily separated. The N-terminal domain of eRF3 is required for prion propagation (7, 8). Point mutations in, or deletion of, the N-terminal domain disrupt the prion propagation function of yeast eRF3, and the N-terminal domain by itself is sufficient for prion propagation (3, 7, 9). The translation termination function of eRF3 requires the well conserved C-terminal domain. Deletion of the C-terminal domain is lethal, presumably because of defects in translation termination, and point mutations in the C-terminal domain reduce translation termination efficiency (9). Thus, the prion property of eRF3 is a property of the N-terminal domain, whereas the C-terminal domain is required for translation termination.

The ability of eRF3 to exist in $[PSI^+]$ and $[psi^-]$ states has been conserved during evolution and is present in the eRF3 proteins of several species and genera of yeast (10–14). The fact that $[PSI^+]$ has been conserved during evolution but that mutations that disrupt it can easily be isolated suggests that there is some evolutionary advantage to $[PSI^+]$.

True and Lindquist (15) showed that $[PSI^+]$ and $[psi^-]$ strains of the same genotype have many different growth phenotypes,

and that the specific phenotypic effects of $[PSI^+]$ depend on the genetic background of the strain used. Most, if not all, of these phenotypic effects are caused by differences in translation termination efficiency, rather than some other effect of PSI state (16). Based on these observations, it has been suggested that the ability to convert from $[PSI^+]$ to $[psi^-]$ and vice versa may aid the evolution of new traits (15). It is currently not known what kinds of target genes are differentially expressed in $[PSI^+]$ versus $[psi^-]$ cells and what the underlying molecular mechanisms are by which $[PSI^+]$ affects expression of these genes.

One mechanism by which $[PSI^+]$ state might affect phenotypes is by causing translational read-through of premature stop codons. Sequence analysis has led to the identification of possible target genes that contain premature stop codons in the one sequenced strain (17, 18). If $[PSI^+]$ causes phenotypes mostly by suppressing premature stop codons, then the strain specific consequences would be caused by different premature stop codons in laboratory strains of yeast. Consistent with the hypothesis that $[PSI^+]$ induces phenotypic variation by suppressing premature stop codons, it has been reported that in seven cases the effect of $[PSI^+]$ was similar to the effect of $upf1\Delta$, which also suppresses premature stop codons (see below) (16). However, it was also reported that in four other cases the effect of $[PSI^+]$ was not mimicked by $upf1\Delta$, suggesting that there may be additional mechanisms by which $[PSI^+]$ affects phenotypes (16).

Two lines of evidence suggest that $[PSI^+]$ affects translation termination not only at premature stop codons, but also at wild-type stop codons naturally found at the 3' end of coding regions. First, the frequency of extra stop codons just 3' of the normal stop codon of yeast genes is higher than expected, suggesting that read-through of the normal stop codons occurs at a frequency that is a significant factor over evolutionary time (19). Second, Namy *et al.* (20) identified eight genes that had a poor stop codon context. In an artificial reporter gene, translation termination at these stop codon contexts was affected by $[PSI^+]$. Thus, $[PSI^+]$ might affect phenotypic variation by promoting read-through of normal stop codons (16–18, 20).

In addition to PSI directly affecting translation termination of premature and/or normal stop codons, there may be indirect effects on mRNA stability because translation termination and mRNA stability are intimately linked (16). Two aspects of this link are nonsense-mediated mRNA decay and nonstop mRNA decay. Nonsense-mediated mRNA decay is the process of rapidly degrading mRNAs that contain a premature stop codon (reviewed in ref. 21). It is thought that nonsense-containing mRNAs are recognized as a consequence of premature translation termination. Because $[PSI^+]$ affects the efficiency of translation termination, it may also affect nonsense-mediated mRNA decay. Nonstop mRNA decay is the process of degrading mRNAs that do not contain stop codons (22, 23). Nonstop mRNAs are

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Abbreviations: ADE, adenine; SC, synthetic complete medium; YCB, yeast carbon base; YEP, yeast extract/peptone; YPD, yeast extract/peptone/dextrose.

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Table 1. Strains used

Strain	Genotype	Source
5V-H19A	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, [PSI ⁺]	Ref. 9
yAV617	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>upf1Δ::KanMX4</i> , [PSI ⁺]	This study
yAV621	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>ski7Δ::KanMX4</i> , [PSI ⁺]	This study
yAV703	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, <i>sup35-ΔN</i> , [psi ⁻]	This study
yAV705	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, <i>sup35-ΔN</i> , <i>upf1Δ::KanMX4</i> , [psi ⁻]	This study
yAV707	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, <i>sup35-ΔN</i> , <i>ski7Δ::KanMX4</i> , [psi ⁻]	This study
yAV734	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>ski2Δ::KanMX4</i> , [PSI ⁺]	This study
yAV736	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>ski3Δ::KanMX4</i> , [PSI ⁺]	This study
yAV738	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>ski8Δ::KanMX4</i> , [PSI ⁺]	This study
yAV740	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>ski7-ΔC::KanMX4</i> , [PSI ⁺]	This study
yAV831	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, [psi ⁻]	This study
yAV833	<i>mata</i> , <i>ade2-1</i> , <i>can1-100</i> , <i>leu2-3,112</i> , <i>ura3-52</i> , SUP16, SUP35, <i>ski7Δ::KanMX4</i> , [psi ⁻]	This study

thought to be recognized when a ribosome reaches the 3' end of an mRNA. In the [PSI⁺] state, ribosomes may reach the 3' end of a normal mRNA after reading through one or more stop codons and may trigger nonstop mRNA decay of such normal mRNAs. Importantly, even a single ribosome that reaches the 3' end of an mRNA is predicted to trigger nonstop decay and reduce the level of mRNA available for translation. Thus, the [PSI⁺] state may affect both nonsense-mediated and nonstop decay of some mRNAs, which in turn could affect phenotypic variation.

To examine how PSI status interacts with nonsense-mediated decay and nonstop decay, we examined [PSI⁺]-induced phenotypic variation in strains defective in either of these mRNA decay pathways. Strikingly, we identified a clear effect of defects in nonstop mRNA decay on [PSI⁺]-induced phenotypic variation. These results suggest that read-through of "normal" stop codons, which produces extended proteins, may be an additional mechanism that contributes to the phenotypic effects of [PSI⁺] and that, at least in some cases, nonstop mRNA decay limits [PSI⁺]-induced phenotypic variation. These results are consistent with the hypothesis that the [PSI⁺] state allows yeast to periodically explore 3' UTR sequences for novel adaptations (15, 16).

Materials and Methods

Strains. The genotypes of the used strains are listed in Table 1. By sequencing we demonstrated that *ade2-1* is a premature UAA codon at codon 64, and *can1-100* contains a premature UAA stop codon at codon 47 (24).

To ensure that the various strains were completely isogenic, we created various deletions in this [PSI⁺] strain by transformation with gene knockout cassettes. The *ski7-ΔC* gene was introduced similarly by amplifying the *ski7-ΔC::KanMX4* gene (23). All deletions were confirmed by PCR.

Unless otherwise indicated, the [psi⁻] strains used were created by precisely deleting the prion-forming domain (amino acids 1–123) of eRF3. Other methods of creating [psi⁻] variants have the disadvantage that they also cure other yeast prions {e.g., [URE3] and [PIN⁺] (1, 18)}. In addition, successful conversion to [psi⁻] is usually monitored by failure to grow on synthetic complete medium (SC) lacking adenine (ADE), but this phenotype is modified by the *upf1Δ* and *ski7Δ* mutations used in this study (see below). The prion-forming N domain was precisely deleted, using the pop-in, pop-out method with HindIII-cut pAv208, which is a pRS306 derivative that contains the promoter and M domain of the eRF3 gene of strain 5V-H19A (25, 26).

Alternatively, yAV603 and yAV621 were converted to [psi⁻] strains yAV831 and yAV833 by growing them on yeast extract/peptone/dextrose (YPD) plus 5 mM guanidinium hydrochloride, and identifying [psi⁻] variants by their slower growth on SC lack-

ing ADE and faster growth on complete supplement mixture medium lacking arginine and containing canavanine.

Media and Growth. Media were prepared as described in refs. 15 and 27. To test growth on various media, strains were grown in YPD at 30°C, serially diluted in 5-fold steps, plated on various media, and incubated at 30°C with growth monitored daily for up to 14 days.

Northern Blot Analysis. Strains were grown in YPD at 30°C to early to middle log phase. ADE2 RNA was analyzed by using Northern blot, probe oAV92 (tctatgctgttccacagggacatttgg), and a Storm imager (Molecular Dynamics). mRNA levels were corrected for loading by using oRP100 (gtctagccgaggaagg), which detect the signal recognition particle RNA. PGK1pG mRNA was detected by using probe oRP140 after transformation with plasmid pRP469 and growth in SC-URA containing 2% galactose and 2% sucrose (28).

Results

[PSI⁺] and Premature Stop Codons. Previous results suggest that one mechanism by which [PSI⁺] affects phenotype is by allowing read-through of premature stop codons (16). To further test this hypothesis, we first compared the phenotype of a [PSI⁺] strain to the phenotype caused by another method of suppressing premature stop codons. Many mutations can suppress premature stop codons (e.g., *sup35*, *sup45*, and suppressor tRNAs), but most of these are also expected to cause read-through at normal stop codons and are not useful for distinguishing the contribution of read-through at premature versus normal stop codons. In contrast, Upf1p appears to specifically recognize mRNAs containing premature stop codons. Upf1p recognition of an mRNA containing a premature stop codon has been reported to have a variety of effects *in vivo*. One major effect is to specifically stabilize such mRNAs (29). Upf1p may also specifically alter translation termination or translation initiation on these mRNAs (24, 30–33). The net effect of *upf1Δ* is a suppression of the phenotype of premature stop codons. These *in vivo* data are supported by the recent demonstration that in *in vitro* translation extracts the fate of the ribosome is different at premature stop codons as compared with normal stop codons, and that this difference depends on Upf1p (33). Thus, all available data suggest that *upf1Δ* specifically suppresses the phenotype caused by premature stop codons. To analyze the significance of suppression of premature stop codons to PSI variable phenotypes, we compared the effect of [PSI⁺] with the effect of loss of Upf1p.

We first confirmed the suppression of the phenotype of known premature stop codons by [PSI⁺] and *upf1Δ*. As expected, premature stop codons in ADE2 and CAN1 are partially suppressed in both the *UPF1* [PSI⁺] strain and in the *upf1Δ* [psi⁻]

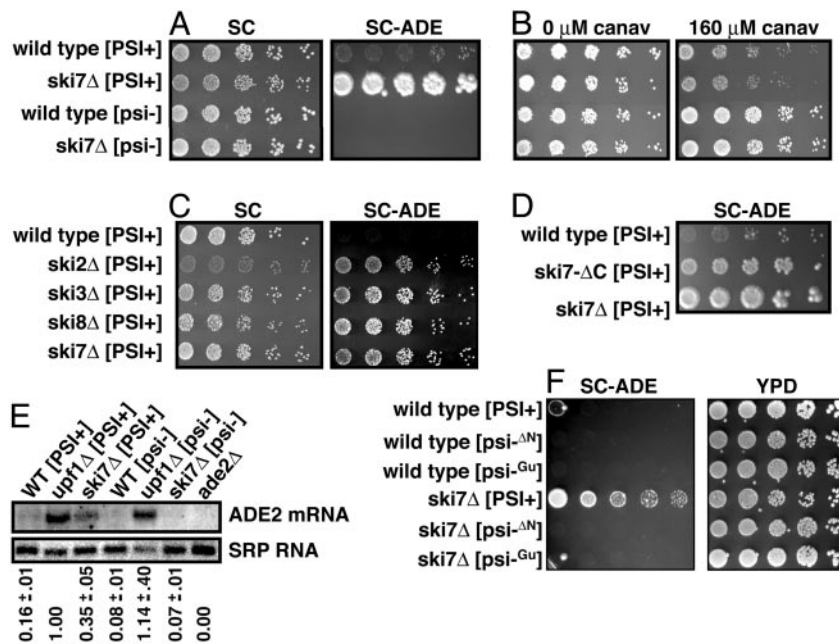


Fig. 3. Defects in nonstop mRNA decay suppress the premature stop codon in *ade2-1* in a $[PSI^+]$ -dependent manner. (A) *ski7Δ* only suppresses *ade2-1* in a $[PSI^+]$ background. (B) *ski7Δ* does not affect *can1-100* suppression. (C) *ski2Δ*, *ski3Δ*, *ski7Δ*, and *ski8Δ* each suppress *ade2-1* in a $[PSI^+]$ strain. (D) A mutation in *SKI7* that inactivates nonstop decay but does not interfere with general exosome-mediated mRNA decay (*ski7-ΔC*) suppresses *ade2-1* in a $[PSI^+]$ strain. (E) The *ade2-1* mRNA level is increased by *ski7Δ* in a $[PSI^+]$ -dependent manner. The numbers in E are the *ade2-1* mRNA levels from two independent experiments, relative to the level in the *upf1Δ* $[PSI^+]$ strain.

nonstop decay (23, 34, 35). We therefore deleted the genes for each of these proteins from the $[PSI^+]$ strain and analyzed their effect on *ade2-1* expression. Fig. 3C shows that each of these deletions increases *ade2-1* expression. Finally, we deleted just the C-terminal domain of Ski7p. This domain is specifically required for nonstop decay and likely functions in the recognition of ribosomes that have reached the 3' end of an mRNA (23). Importantly, this *ski7-ΔC* mutation also results in increased expression of *ade2-1* (Fig. 3D). Thus, analyses of growth phenotype indicate that $[PSI^+]$ genetically interacts with the nonstop mRNA decay pathway to alter *ade2-1* expression.

To confirm that the observed growth phenotypes on SC-ADE indeed reflected *ade2-1* expression, we analyzed the *ade2-1* mRNA by Northern blotting. Fig. 3E shows that the *ade2-1* mRNA level is increased by the *upf1Δ* in either the $[PSI^+]$ or $[psi^-]$ background. This finding is consistent with the increase in growth seen on SC-ADE in Fig. 2A. As expected from the growth phenotype, *ski7Δ* resulted in an increase in *ade2-1* mRNA levels in the $[PSI^+]$ state but had no detectable effect in the $[psi^-]$ state (Fig. 3E). This result is consistent with our interpretation that the $[PSI^+]$ state triggers nonstop mRNA degradation of the *ade2-1* mRNA.

The results above were obtained by using a $[psi^-]$ strain that is lacking the prion-forming N terminus of eRF3. Our data therefore indicate that the N terminus of eRF3 genetically interacts with nonstop decay. The most likely explanation is that the $[PSI^+]$ prion form of the N terminus of eRF3 triggers nonstop decay. Alternatively, the N terminus of eRF3 can physically interact with several other proteins, and one of these physical interactions may be responsible for the genetic interaction with the nonstop mRNA decay pathway (36, 37). To test whether $[PSI^+]$ is responsible for the genetic interaction, we converted $[PSI^+]$ strains to $[psi^-]$ by growing them in the presence of guanidinium HCl (1, 2). Fig. 3F shows that strains that contain the N terminus of eRF3 in the $[psi^-]$ conformation have the same phenotype as strains missing the prion-forming domain

of eRF3. Therefore, we conclude that $[PSI^+]$, and not some other feature of the N terminus of eRF3, genetically interacts with nonstop mRNA decay.

The results above suggest that the $[PSI^+]$ state could trigger read-through of all stop codons in some other mRNAs and thereby reduce their levels in a manner dependent on nonstop decay. To test this prediction, we examined the steady state levels of several other mRNAs in $[PSI^+]$ and $[psi^-]$ strains, with or without a functional nonstop decay system. We found no significant differences in the levels of the RPS15 or RPL25 mRNAs. Strikingly, we observed that the $[PSI^+]$ state showed 2-fold reduced levels of the PGK1pG mRNA and that, in a $[PSI^+]$ *ski7Δ* strain, the mRNA level was increased (Table 2). This result provides additional evidence that the $[PSI^+]$ state can trigger nonstop decay of mRNAs that contain a stop codon. Most importantly, the conclusion that $[PSI^+]$ can trigger decay of mRNAs that contain stop codons in a process that is indistinguishable from nonstop mRNA decay raises the possibility that some of the phenotypic variation caused by $[PSI^+]$ is caused or modulated by nonstop mRNA decay.

Nonstop mRNA Decay Modulates $[PSI^+]$ Phenotypes. To examine whether the genetic interaction between $[PSI^+]$ and *ski7Δ* was specific to *ade2-1* or also affected other phenotypes, we compared the phenotype of wild-type $[PSI^+]$ and $[psi^-]$ strains to isogenic *ski7Δ* strains under 16 different conditions where growth has previously been shown to be affected by $[PSI^+]$

Table 2. Effect of $[PSI^+]$ and *ski7Δ* on PGK1pG mRNA

Strain	PGK1pG mRNA level, %
$[psi^-]$	100
<i>ski7Δ</i> $[psi^-]$	83 ± 15
$[PSI^+]$	55 ± 8
<i>ski7Δ</i> $[PSI^+]$	244 ± 10

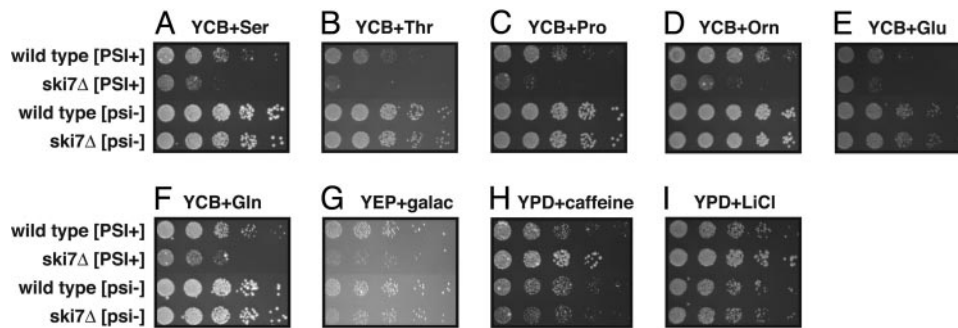


Fig. 4. *ski7Δ* has various phenotypic consequences in a $[PSI^+]$ strain but not in an isogenic $[psi^-]$ strain. (A) YCB plus 1 g/liter serine, 3 days of growth. (B) YCB plus 1 g/liter threonine, 3 days of growth. (C) YCB plus 1 g/liter proline, 3 days of growth. (D) YCB plus 1 g/liter ornithine, 3 days of growth. (E) YCB plus 1 g/liter glutamate, 2 days of growth. (F) YCB plus 1 g/liter glutamine, 3 days of growth. (G) YEP plus 2% galactose, 5 days of growth. (H) YPD plus 10 mM caffeine, 6 days of growth. (I) YPD plus 50 mM LiCl, 3 days of growth.

(15). This experiment showed that nonstop decay modulates the phenotypic effect of $[PSI^+]$. In six conditions, we found results similar to those for *ade2-1*: The phenotypic effect of $[PSI^+]$ is further increased by *ski7Δ* (growth on YCB plus serine, threonine, proline, ornithine, glutamate, or glutamine; Fig. 4 A–F). Under these conditions, $[PSI^+]$ isolates grow slower than $[psi^-]$ isolates (compare first and third rows). Deletion of *SKI7* further decreases growth in the $[PSI^+]$ state (compare first and second rows) but has no effect in the $[psi^-]$ state (compare third and fourth rows). Thus, in some cases the capacity of $[PSI^+]$ to reveal new phenotypes is limited by nonstop decay. Additional examples of this limiting effect can be seen on YEP plus galactose, YPD plus caffeine, and YPD plus LiCl. In these three cases, wild-type $[PSI^+]$ and $[psi^-]$ strains have (almost) the same phenotype (Fig. 4 G–I, first and third rows), but a clear difference can be seen between *ski7Δ* $[PSI^+]$ and *ski7Δ* $[psi^-]$ strains (second and fourth rows). Under the remaining conditions, we did not see a clear reproducible difference between the phenotypes of $[PSI^+]$ and $[psi^-]$ strains, or the difference was not affected by *ski7Δ* (Fig. 5 and data not shown). Thus, in 9 of 16 conditions examined, *ski7Δ* affects the phenotype in the $[PSI^+]$ state but has no effect in the $[psi^-]$ state. We conclude that the genetic interaction between nonstop mRNA decay and $[PSI^+]$ is not limited to *ade2-1* and that nonstop mRNA decay may, in some cases, limit the capacity of $[PSI^+]$ to reveal new phenotypes.

Discussion

$[PSI^+]$ Genetically Interacts with Nonstop mRNA Decay. We have shown a genetic interaction between $[PSI^+]$ and mutations that inactivate the nonstop mRNA decay pathway. The central observation is that strains defective in nonstop mRNA decay show a greater variation in phenotype between $[psi^-]$ and $[PSI^+]$ states than corresponding wild-type $[psi^-]$ and $[PSI^+]$ pairs. This genetic interaction affects the expression of *ade2-1* and growth under 9 of 16 conditions tested. It is unclear whether these nine phenotypes reflect read-through events on different mRNAs, or whether several of these phenotypes are caused by the same read-through event of the same mRNA. In either case, a simple explanation for this interaction is that $[PSI^+]$ causes read-through of normal stop codons and any other stop codons in the 3' UTR. Such read-through would cause a ribosome to reach the very 3' end of the mRNA and would trigger nonstop mRNA decay (22, 23). This possibility is supported by the observation that $[PSI^+]$ leads to a Ski7p-dependent reduction in the levels of the PGK1 and *ade2-1* mRNAs (Fig. 3 and Table 2). Because yeast 3' UTRs are relatively short, with many shorter than 100 nt, the ribosome would only have to read through one or two additional stop codons in the 3' UTR to reach the 3' end of such

mRNAs (38). Thus, $[PSI^+]$ may trigger some degree of nonstop mRNA decay on a variety of different mRNAs.

The ability of $[PSI^+]$ to induce nonstop mRNA decay of specific mRNAs is affected by several factors. One likely factor is the identity and sequence context of the stop codons, which can affect the degree of suppression by $[PSI^+]$ (16). Another likely contributor is the number and type of in-frame stop codons within the 3' UTR. These additional factors may explain why the *ade2-1* allele is affected strongly by $[PSI^+]$ *ski7Δ*, whereas the *can1-100* allele is less affected. In the *ade2-1* mRNA, the premature stop codon, the normal stop codon, and the next stop codon in the 3' UTR are all UAA codons. In contrast, the *can1-100* mRNA contains a UAA premature stop codon, a UAG at the end of the natural ORF, and a UGA in the 3' UTR. Although the read-through frequency of these stop codons within *ade2-1* and *can1-100* is unknown, the read-through frequency of UAA, UAG, and UGA codons in the $[PSI^+]$ 5V-H19A strain has been measured (16). Using these frequencies, the combination of the normal UAG codon and UGA at +2 in *can1-100* is expected to be read through 33 times less efficiently than the combination of the normal UAA codon and the UAA codon at +12 in *ade2-1*. This analysis suggests that one possible reason for the difference in effects of *ski7Δ* may be that the combination of stop codons in *ade2-1* is read through more efficiently than that in *can1-100*, and thus the ribosome is more likely to reach the 3' end of the *ade2-1* mRNA. This interpretation implies that the ability of $[PSI^+]$ to read through normal stop codons and/or trigger nonstop mRNA decay on individual mRNAs will vary significantly.

Significance of Read-Through of Normal Stops and Nonstop mRNA Decay for $[PSI^+]$ -Induced Phenotypes. We can envision two possible mechanisms by which translation past the normal stop codon and into the 3' UTR may affect the phenotype. First, translation all of the way through the 3' UTR should trigger nonstop mRNA decay and thereby reduce the level of protein that can be made from that mRNA by subsequent rounds of translation. This mechanism is inconsistent with our findings, because it predicts that $[PSI^+]$ and $[psi^-]$ phenotypes are the same in a strain that is incapable of nonstop mRNA decay (e.g., *ski7Δ*). Thus, triggering nonstop decay *per se* is not a main mechanism that explains the phenotypic consequences of $[PSI^+]$ that we observe. In an alternative model, translation into the 3' UTR produces C-terminally extended proteins. Our observation that *ski7Δ* enhances $[PSI^+]$ phenotypes is consistent with this hypothesis, because *ski7Δ* would increase the level of target mRNAs and, in turn, increase production of the C-terminally extended proteins encoded by that mRNA. A corollary of this interpretation is that the C-terminally extended proteins are functional and actively contribute to the phenotype of $[PSI^+]$ strains.

A final understanding of all of the molecular mechanisms behind each of the $[PSI^+]$ -induced phenotypes requires identification of individual target genes. Our results suggest that one way to find some of these target genes is by microarray analyses on $[PSI^+]$ versus *ski7Δ* $[PSI^+]$. Our results also indicate that a bioinformatic approach to finding target genes may be more successful if it takes into account the presence of additional stop codons within the 3' UTR. It should also be noted that we examined phenotypic variation in strain 5V-H19A, because in this strain $[PSI^+]$ is especially effective at modifying growth phenotypes. This strain contains a weak suppressor tRNA (*SUP16*). Thus, we cannot rule out the formal possibility that the genetic interaction we observe requires the suppressor tRNA. However, this seems unlikely because $[PSI^+]$ induces efficient read-through of stop codons and phenotypic variation even in the absence of suppressor tRNAs (15, 39).

Multiple Mechanisms of $[PSI^+]$ -Induced Phenotypic Variation. Earlier work by True *et al.* (16) showed that in 7 of 11 cases examined, $[PSI^+]$ -induced phenotypic variation could be mimicked by *upf1Δ*, which affects nonsense-mediated decay and allows suppression of premature stop codons. This finding suggests that, in some cases, $[PSI^+]$ -induced phenotypic variation is caused by the suppression of premature stop codons. However, True *et al.* (16) also observed that some phenotypes were not mimicked by *upf1Δ*, suggesting there are additional manners by which $[PSI^+]$ affects phenotypic variation. Consistent with this latter conclusion, we also observed different phenotypic consequences for $[PSI^+]$ and *upf1Δ* in our experiments. Thus, the data presented by True *et al.* and us suggest that $[PSI^+]$ affects gene expression by at least two distinct mechanisms. First, $[PSI^+]$ causes read-through of premature stop codons, thereby restoring protein production. Second, $[PSI^+]$ causes read-through of normal stop

codons, thereby possibly producing C-terminally extended proteins. Database searches for premature stop codons and normal stop codons in poor context have identified a number of putative $[PSI^+]$ target genes (17, 18, 20). However, True *et al.* have shown that most of the phenotypic consequences of $[PSI^+]$ are multigenic. This finding suggests that $[PSI^+]$ -induced phenotypic variation will involve changes in the expression of multiple mRNAs. Thus, a single $[PSI^+]$ -induced phenotypic difference could be due to a combination of both the read-through of premature and normal stop codons.

Implications for a Role for $[PSI^+]$ in Evolution. The possible exploration of 3' UTRs for advantageous encoded protein sequences may have advantages as an evolutionary mechanism (15, 16). In the $[psi^-]$ state the 3' UTR could accumulate sequence variation with little selective pressure. Indeed, comparison of genome sequences of closely related yeast species shows that 3' UTR sequences are less conserved than coding regions or promoters (40, 41). An occasional switch to the $[PSI^+]$ state would allow the strain to explore the accumulated diversity for advantageous proteins. If any of the newly translated sequences offered an advantage, this advantage could, over time, become independent of $[PSI^+]$, by mutation of a stop codon to a sense codon. Subsequently, the cell could revert to $[psi^-]$ without losing the novel adaptation, while losing other C-terminally extended proteins that may be deleterious. The advantage of this mechanism may be further increased by preferentially switching to $[PSI^+]$ under severely adverse conditions, which can affect the conversion of $[psi^-]$ to $[PSI^+]$ (18, 42).

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