Oxidation of phenylalanyl-tRNA synthetase positively regulates translational quality control

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Accurate translation of the genetic code is maintained in part by aminoacyl-tRNA synthetases (aaRS) proofreading mechanisms that ensure correct attachment of a cognate amino acid to a transfer RNA (tRNA). During environmental stress, such as oxidative stress, demands on aaRS proofreading are altered by changes in the availability of cytoplasmic amino acids. For example, oxidative stress increases levels of cytotoxic tyrosine isomers, noncognate amino acids normally excluded from translation by the proofreading activity of phenylalanyl-tRNA synthetase (PheRS). Here we show that oxidation of PheRS induces a conformational change, generating a partially unstructured protein. This conformational change does not affect Phe or Tyr activation or the aminoacylation activity of PheRS. However, in vitro and ex vivo analyses reveal that proofreading activity to hydrolyze Tyr-tRNA^{Phe} is increased during oxidative stress, while the cognate Phe-tRNA^{Phe} aminoacylation activity is unchanged. In HPX^{-}, *Escherichia coli* that lack reactive oxygen-scavenging enzymes and accumulate intracellular H_2O_2, we found that PheRS proofreading is increased by 11%, thereby providing potential protection against hazardous cytoplasmic *m*-Tyr accumulation. These findings show that in response to oxidative stress, PheRS proofreading is positively regulated without negative effects on the enzyme's housekeeping activity in translation. Our findings also illustrate that while the loss of quality control and mistranslation may be beneficial under some conditions, increased proofreading provides a mechanism for the cell to appropriately respond to environmental changes during oxidative stress.

aminoacyl-tRNA | oxidative stress | proofreading | quality control

Translation of the genetic code is an essential process that generates functional proteins in the cell. One key player in translation is the aminoacyl-tRNA synthetase, which canonically ligates an amino acid to its cognate tRNA at the 3′CCA end of the acceptor stem (1). Aminoacylated tRNA (aa-tRNA) is used to decode mRNA at the ribosome to synthesize peptides in a codon-specific manner. In the case of phenylalanyl-tRNA synthetase (PheRS), phenylalanine (Phe) is aminoacylated onto tRNA^{Phe} to generate Phe-tRNA^{Phe}. Many amino acids have similar chemical and physical properties. Therefore, aaRSs must be able to discriminate against noncognate amino acids to ensure accurate translation of the genetic code. To aid in this, some aaRS, including PheRS, have proofreading activity to maintain high accuracy (2, 3). Proofreading can occur before the amino acid is ligated onto a tRNA or after the ester linkage has been formed, termed pretransfer and posttransfer editing, respectively (3). For example, tyrosine (Tyr) and Phe differ in structure by one hydroxyl group; therefore, PheRS uses its proofreading activity to discriminate against ligating Tyr onto tRNA^{Phe}. If Tyr-tRNA^{Phe} goes to the ribosome, Tyr can be misincorporated into the growing peptide at Phe codons, causing mistranslation. Translational errors can lead to the formation of protein aggregates, which have been implicated in numerous physiological defects (4–6). Therefore, maintaining translational accuracy is an essential cellular process.

Oxidative stress, an imbalance in the levels of free oxygen and nitrogen radicals, has previously been documented to alter translational fidelity and can have adverse effects on the cell if not properly regulated (5, 7). DNA, RNA, and proteins can be affected by oxygen radicals independently; therefore, all steps of translating the genetic code are susceptible to oxidative stress (8–12). Amino acid oxidation can cause changes to proteins that allow different interactions with the environment and altered activity (13). Oxidative stress causes diverse effects on aaRSs in various organisms. For example, in the case of *Escherichia coli* threonyl-tRNA synthetase (ThrRS), oxidation of a critical cysteine in the editing domain leads to reversible inactivation of editing, allowing for serine (Ser) to be misacylated onto tRNA^{Thr}. Accumulation of Ser-tRNA^{Thr} in the cell has potential to cause mistranslation at Thr codons (14). Conversely, in yeast, reversible oxidation of the mitochondrial PheRS inactivates aminoacylation activity due to the formation of disulfide bonds (15). This inactivation of PheRS leads to a growth defect in yeast, revealing that the aminoacylation activity of mitochondrial PheRS is critical for yeast during oxidative stress. In the case of mammalian methionyl-tRNA synthetase (MetRS), during oxidative stress, phosphorylated MetRS is able to interact with other noncognate tRNAs. MetRS is able to aminoacylate many tRNAs in the cells during oxidative stress to increase the presence of Met in cellular proteins (16). Increasing the proportion of Met in peptides protects the rest of the proteome from oxidative damage by using the additional sulfur groups to act as sinks for oxygen radicals. These examples highlight the diverse effects of oxidative stress on enzymatic activity and substrate specificity of aaRSs during translation.

Salmonella enterica serovar Typhimurium (*S*. Typhimurium) is an organism that is transiently exposed to high levels of oxidative stress. It is able to adapt to the acidic environment in the gastrointestinal tract, outcompete bacteria that typically reside in the gut, and cause infection where other bacteria are often removed by the immune

Significance

Regulation of translational quality control during adverse growth conditions is critical to maintain cellular homeostasis. Translational quality control is maintained by proofreading by the aminoacyl-tRNA synthetases (aaRS), which ensure that cognate aminoacyl-tRNAs are provided to the ribosome for protein synthesis. Our findings now show that under oxidative stress a global conformational change in the phenylalanyl-tRNA synthetase positively regulates quality control by increasing the rate of proofreading. These results demonstrate that the translational machinery is able to rapidly and positively respond to environmental challenges to maintain the accuracy of protein synthesis.

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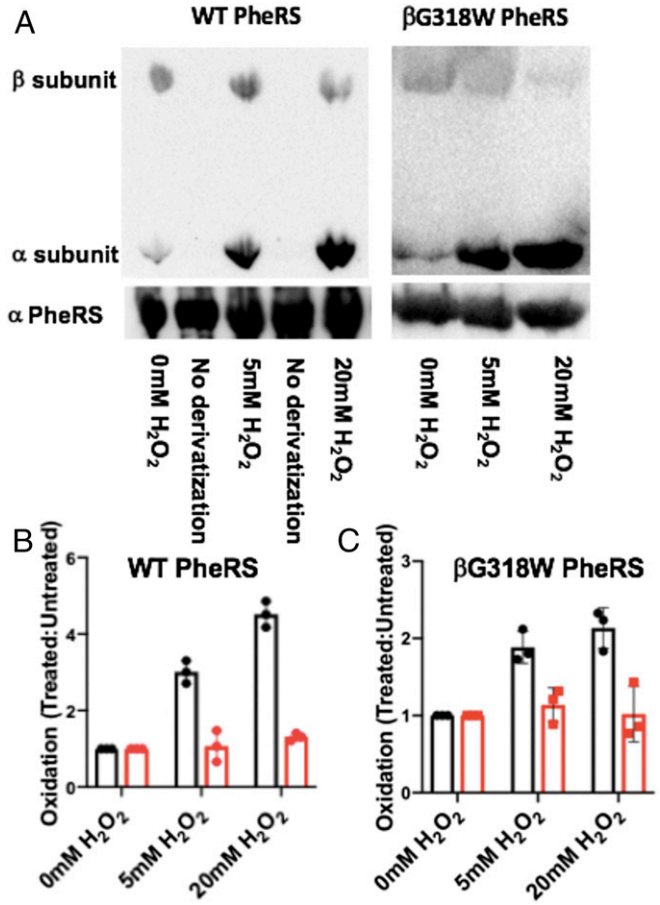

Fig. 1. Quantification of oxidation of recombinant WT and editing-deficient PheRS. (*A*) A representative Western blot showing the oxidation of the α- and β-subunits of WT and βG318W PheRS. Lanes labeled no derivatization serve as a control to ensure the protein does not interact with the antibody unless derivatized with the aromatic structure. (*B*) Quantification of the relative oxidation of WT PheRS with 5 and 20 mM H_2O_2 relative to 0 mM H_2O_2. (*C*) Quantification of the relative oxidation of βG318W PheRS with 5 and 20 mM H_2O_2 relative to 0 mM H_2O_2. α-subunit quantified (black) and β-subunit quantified (red).

response (17). *Salmonella* survives within the host microbiome in part due to its resiliency against oxidative stress generated during the host's immune response. One method by which *Salmonella* survives is through its ability to replicate within a macrophage, an immune cell that generates reactive oxygen species (ROS) (18). It has previously been shown that ROS can target free amino acids, generating imbalances to the endogenous cellular amino acid pool (7). Specifically, during oxidative stress, there is an increase in the presence of *ortho, meta*, and *para*-Tyr isomers due to Phe oxidation at various positions of the aromatic ring (7). The generation of these isomers alters the amino acid pools, and high concentrations of noncognate amino acids can lead to mistranslation, presenting a significant challenge for PheRS proofreading. Therefore, understanding how PheRS compensates for these changes in the environment will explain how translational accuracy is affected. Here we report that during oxidative stress, *S*. Typhimurium PheRS undergoes a conformational change that allows aminoacylation activity to be retained while simultaneously increasing proofreading activity, preventing the accumulation of *m*-Tyr-tRNA^{Phe}. In vitro and ex vivo analyses reveal that oxidative stress generates a hyperaccurate PheRS, positively regulating translational quality control during oxidative stress.

Results

***Salmonella* PheRS Is Subject to Protein Oxidation.** To investigate the possible role of PheRS in maintaining translational fidelity during oxidative stress, a recombinant PheRS was purified and used for subsequent in vitro analysis. *S*. Typhimurium and *E. coli* share a 95% sequence identity; therefore, a mutation in the β-subunit of PheRS (βG318W) was made in *S*. Typhimurium at the same position previously used to generate an editing-deficient *E. coli* PheRS (2). Editing-deficient *S*. Typhimurium PheRS was confirmed by its inability to hydrolyze Tyr-tRNA^{Phe} (*SI Appendix*, Fig. S1). Using WT and editing-deficient *S*. Typhimurium PheRS, the role of translational fidelity during oxidative stress was further examined. Upon exposure to oxidative stress, many residues in both the WT and editing-deficient PheRS become oxidized. Immunoblot analysis, probing for 2,4-dinitrophenylhydrazine (DNPH) derivatized residues, indicated that PheRS oxidation seems to occur primarily in the α-subunit of PheRS, which includes the catalytic active site (Fig. 1). The β-subunit of PheRS, which contains the editing site, did not show significant oxidation after 5 or 20 mM H_2O_2 treatment (Fig. 1). This immunoblotting technique with anti-DNPH is only able to indicate ketones and aldehydes as oxidized residues; therefore, not all amino acid oxidation can be detected using these results. We used mass spectrometry and 5–5′-dithio-bis-[2-nitrobenzoic acid] (DTNB) analysis to further determine the extent of PheRS oxidation upon H_2O_2 treatment. Mass spectrometry analysis using oxidized recombinant proteins revealed extensive oxidation in both the WT and editing-deficient PheRS, with different residues being oxidized in each case (*SI Appendix*, Table S1). In addition, DTNB analysis revealed that the WT and editing-deficient PheRS have different oxidation patterns (Table 1). The WT PheRS is accessible to DTNB, while the editing-deficient PheRS does not show reactivity with DTNB, even without treatment with H_2O_2. Treatment of editing-deficient PheRS with 6 M guanidine hydrochloride to unfold the protein makes all cysteines accessible to the DTNB reagent. Combined, these results reveal that the oxidation of *S*. Typhimurium PheRS has a global effect on the structure of the protein beyond local oxidation of a single amino acid.

Protein Oxidation Causes a Conformational Change of PheRS. Protein oxidation can lead to the formation of disulfide bonds and other amino acid changes that induce a conformational change (15, 19). To investigate why editing-deficient and WT PheRS have different oxidation patterns, circular dichroism was used to explore the possibility of a conformational change in the editing-deficient PheRS. Circular dichroism reveals information about the secondary structure of proteins, indicating if a protein is mostly composed of β-sheets, α-helices, or is unstructured. Editing-deficient PheRS has an increase in the α-helical content compared with the WT PheRS, indicating more intrinsic structure (Fig. 2*A*). This increase in structure is consistent with mass spectrometry and DTNB analysis in that a more structured protein is not oxidized to the same extent as the WT PheRS. In addition, circular dichroism revealed that oxidation of both WT and editing-deficient PheRS decreases the α-helical content of the protein (Fig. 2*B*). Oxidation of PheRS causes a global conformational change to the protein,

Table 1. DTNB reactivity with cysteine

Protein	Number of Cysteines
WT PheRS	5
WT PheRS + 5 mM H_2O_2	1
Edt (–) PheRS	1
Edt (–) PheRS + 5 mM H_2O_2	1
Edt (–) PheRS + 6M GndHCl	4

Number of cysteines that react with DTNB reagent based on luminescence quantification.

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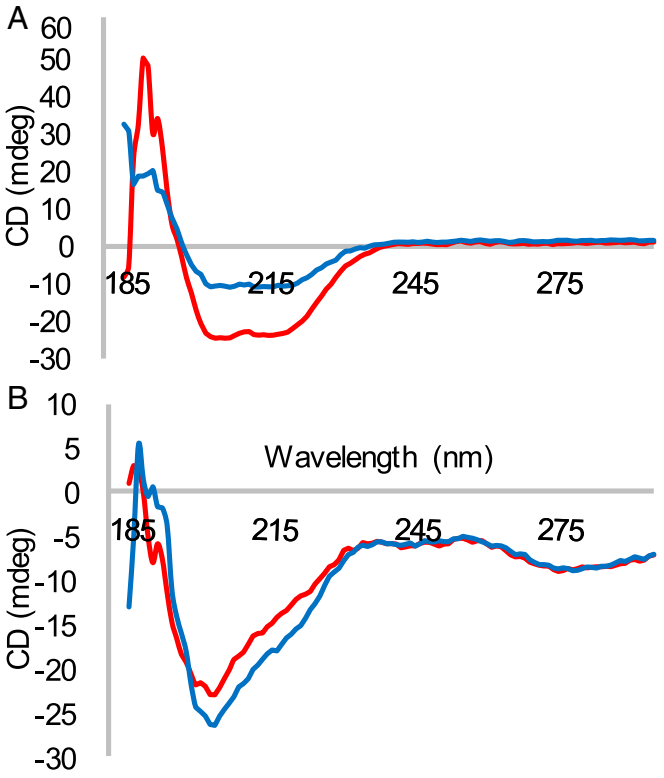

Fig. 2. (*A*) Circular dichroism of recombinant WT (blue) and βG318W PheRS (red) between 200 and 285 nm. (*B*) Circular dichroism of oxidized recombinant WT (blue) and oxidized recombinant βG318W PheRS (red) between 200 and 285 nm.

modifying its structure, which in turn could impact the activity of PheRS.

Aminoacylation Activity of PheRS Is Not Affected by Oxidative Stress. To determine if the conformational change induced by oxidative stress alters PheRS activity, in vitro analyses were performed. It has previously been reported that a change in a protein's structure may correlate with a change in enzymatic function. For example, RfaH exists in two forms, in which the C-terminal domain is made up of α-helices or β-sheets (20). These two different conformational states are either autoinhibitory or translational activating, respectively, highlighting the potential of one protein to play two distinct roles based on its secondary structure (20). Steady-state kinetics of amino acid activation were determined by pyrophosphate exchange, revealing that oxidative stress does not impact the K_M for activation of cognate Phe or the noncognate *m*-Tyr (Table 2) (2). In addition, tRNA aminoacylation reactions were used to determine the K_M of $tRNA^{Phe}$, which also revealed no significant changes during oxidative stress (Table 2). These results indicate that the conformational change caused by oxidation of PheRS does not impact PheRS activation or cognate aminoacylation of $tRNA^{Phe}$.

Oxidation of PheRS Increases Noncognate Substrate Proofreading. We have previously demonstrated that oxidative stress leads to elevated cytoplasmic levels of Tyr isomers, with *m*-Tyr accumulating to levels that lead to cytotoxic mistranslation when PheRS editing activity is impaired (7). To elucidate the effect of oxidative stress on proofreading activity of PheRS, the hydrolysis of misacylated *p*-Tyr-$tRNA^{Phe}$ was investigated (Fig. 3). In the case of the WT PheRS, treatment of PheRS with H_2O_2 increases the ability to hydrolyze mischarged $tRNA^{Phe}$ but does not impact the half-life of Tyr-$tRNA^{Phe}$ (Fig. 3 and *SI Appendix*, Fig. S2 and Table S2). Editing-deficient PheRS, upon treatment with H_2O_2, partially regains the ability to proofread mischarged *p*-Tyr-$tRNA^{Phe}$ by increasing hydrolysis of the ester linkage. In addition, oxidized editing-deficient PheRS decreases the half-life of Tyr-$tRNA^{Phe}$ by one-half of the nonoxidized editing-deficient PheRS (Fig. 3 and *SI Appendix*, Fig. S2 and Table S2). A lower half-life of mischarged $tRNA^{Phe}$ indicates more proofreading occurring. To further analyze the change in proofreading activity of oxidized PheRS, an ATP consumption analysis was performed to quantify the hydrolysis of misacylated $tRNA^{Phe}$. In this analysis, ATP consumption corresponds to the combined activity of pretransfer and posttransfer editing due to the futile cycling of ATP through the reactivation of the hydrolyzed substrate. Consistent with the deacylation experiments, protein oxidation causes both WT and editing-deficient PheRS to consume more ATP after 30 min, which is indicative of an increase in pretransfer and posttransfer editing (Fig. 4*A*). The average rate of ATP consumed between 1 and 10 min revealed that the rate for oxidized and nonoxidized WT PheRS remained similar, with oxidized WT PheRS tending to have a higher rate of ATP consumption by 0.01 mM/min (*SI Appendix*, Table S3). The average rate of ATP consumed increased from 0.02 to 0.03 mM/min between 1 and 10 min when using oxidized βG318W PheRS compared with nonoxidized βG318W PheRS (*SI Appendix*, Table S3). This increase in proofreading activity is specific to misacylated $tRNA^{Phe}$ as Phe-$tRNA^{Phe}$ does not appear to be affected as extensively by H_2O_2 treatment (Fig. 4*B*). The slight increase in ATP consumed during Phe-$tRNA^{Phe}$ proofreading is not significant (*SI Appendix*, Table S4). Additionally, the increase in ATP consumption during oxidative stress is in a tRNA-independent manner (*SI Appendix*, Fig. S3). These results indicate that during oxidative stress, PheRS increases proofreading activity, while maintaining normal aminoacylation activity.

Misaminoacylation of $tRNA^{Phe}$ Decreases During Oxidative Stress. An increase in PheRS proofreading activity during oxidative stress was further quantified by analyzing misacylation of $tRNA^{Phe}$ with *m*-Tyr using TLC. Without H_2O_2 treatment, editing-deficient PheRS is able to misacylate $tRNA^{Phe}$ with noncognate *m*-Tyr to a comparable extent to cognate Phe (*SI Appendix*, Fig. S4 *A* and *B*). Following protein oxidation, the ability of editing-deficient PheRS to mischarge $tRNA^{Phe}$ was decreased by ∼30% (*SI Appendix*, Fig. S4 *A* and *B*). Again, the aminoacylation of Phe-$tRNA^{Phe}$ was not affected by oxidative stress, as this editing activity is specific for noncognate misaminoacylation and does not perturb cognate aminoacylation activity (*SI Appendix*, Fig. S4*C*). These results further

Table 2. Steady-state kinetics for PheRS under oxidative stress

	Phe			*m*-Tyr			$tRNA^{Phe}$		
Enzyme	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (s-1/μM)	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (s-1/μM)	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (s-1/μM)
WT PheRS	35 ± 10	1 ± 0.2	0.03	147 ± 17	0.5 ± 0.1	0.003	1.9 ± 0.7	6.3 ± 0.8	3.3
WT PheRS + 5 mM H_2O_2	40 ± 14	0.8 ± 0.2	0.02	153 ± 31	0.3 ± 0.1	0.002	1.4 ± 0.7	7.2 ± 1	5.1
Edit (–) PheRS	30 ± 7	1.3 ± 0.3	0.04	153 ± 33	0.1 ± 0.01	0.001	2.7 ± 0.4	7.1 ± 2	2.6
Edit (–) PheRS+ 5 mM H_2O_2	30 ± 9	0.6 ± 0.3	0.02	100 ± 31	0.2 ± 0.1	0.003	1.5 ± 0.7	2.4 ± 0.2	1.7

Steady-state kinetic parameters of PheRS for the activation of Phe and *m*-Tyr using ATP-PPi exchange and $tRNA^{Phe}$ using aminoacylation assays.

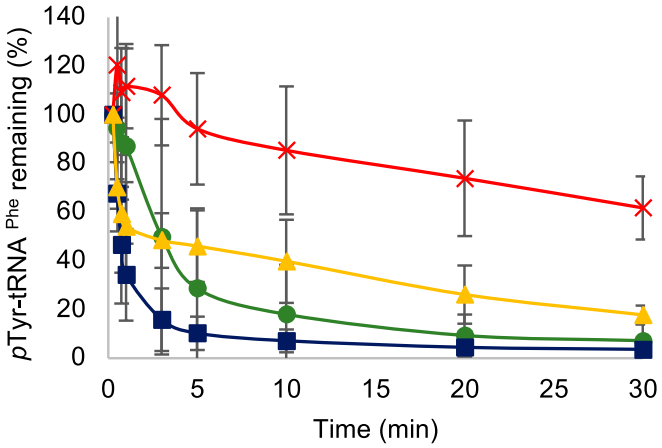

Fig. 3. Hydrolysis of *p*-Tyr-tRNA^{Phe} over time shown by percent of mischarged tRNA^{Phe} remaining. Editing-deficient PheRS (red), editing-deficient PheRS + 5 mM H_2O_2 (yellow), WT PheRS (green), WT PheRS + 5 mM H_2O_2 (blue).

confirm that PheRS is able to increase its fidelity during oxidative stress, while not impacting canonical aminoacylation activity.

***E. coli* PheRS Proofreading Increases During Oxidative Stress in Cell Lysates.** In vitro analyses revealed that treating recombinant PheRS with H_2O_2 leads to increased proofreading activity of PheRS. To investigate if this also happens ex vivo, *E. coli* cell lysates were used in deacylation assays. Midlog-phase liquid cultures of WT and editing-deficient strains of *E. coli* were treated with 20 mM H_2O_2, and cell-free lysates were prepared and assayed for aminoacylation and editing activities. The specific activity of PheRS in the oxidized cell-free lysate was significantly lower than nonoxidized cell lysates (*SI Appendix*, Table S5). Therefore, proofreading activity of PheRS was normalized to the specific activity of aminoacylation using the cell lysates. Using *E. coli* cell-free lysates in editing reactions containing misacylated *p*-Tyr-tRNA^{Phe} revealed that oxidation leads to an increase in editing of misacylated tRNA^{Phe} (Fig. 5*A*), without hydrolyzing cognate-aminoacylated Phe-tRNA^{Phe} (Fig. 5*B*). The half-life of Tyr-tRNA^{Phe} was also quantified to compare oxidative stress to normal conditions. The WT *E. coli* in the presence and absence of H_2O_2 have similar half-life of 11 min (*SI Appendix*, Fig. S5 and Table S6). Whereas the editing-deficient *E. coli* cell lysate under oxidative stress significantly decreases the half-life of mischarged tRNA^{Phe} by 10 min (*SI Appendix*, Fig. S5 and Table S6), the half-life of Phe-tRNA^{Phe} remains unchanged in the presence and absence of H_2O_2 (*SI Appendix*, Fig. S6 and Table S7). These results indicate that translational quality control is maintained during oxidative stress by increasing the proofreading of misacylated tRNA.

***E. coli* Lacking ROS Scavenging Display Increased PheRS Proofreading.** The oxidative stress response in *E. coli* involves the scavenging of oxidative radicals by catalases and peroxidases to break down H_2O_2 into water and oxygen. Without scavenging enzymes, *katG, katE*, and *ahpCF*, oxidative stress levels in the cell increase through the accumulation of H_2O_2 (21). When grown anaerobically, WT *E. coli* accumulates low levels of oxygen radicals, while HPX$^-$ *E. coli* (Δ*katG katE ahpCF*) accumulates high levels of oxygen radicals, due to the lack of scavenging activity (21). Comparing PheRS activities of WT MG1655 and HPX$^-$ *E. coli* cell-free lysates reveals that basal oxidative stress levels have a similar effect compared with growth in the presence of H_2O_2. The specific activity of PheRS in the HPX$^-$ *E. coli* cell-free lysate was lower than WT *E. coli* cell lysates (*SI Appendix*, Table S8). Therefore, proofreading activity was normalized to the specific activity of the cell-free lysates. Using HPX$^-$ *E. coli*, *we* demonstrate that increased H_2O_2 in the cell, through the lack of scavenging enzymes, generates a hyperaccurate PheRS with an increased rate of hydrolysis of *p*Tyr-tRNA^{Phe} (Fig. 6*A* and *SI Appendix*, Fig. S7 and Table S9). The half-life of Tyr-tRNA^{Phe} is significantly lower in the HPX$^-$ *E. coli* compared with WT *E. coli*, indicating an increase in proofreading activity in these cells. Again, the Phe-tRNA^{Phe} half-life does not seem to change between HPX$^-$ and WT *E. coli* (*SI Appendix*, Fig. S8 and Table S10). The extent of increased proofreading is not as high as for cells grown with H_2O_2, consistent with reduced accumulation of H_2O_2 in cells grown anaerobically. Overall, increased proofreading in *E. coli* cell-free lysate lacking scavenging enzymes reveals that even low levels of H_2O_2 produced by the cell have the potential to increase PheRS accuracy.

Discussion

PheRS Oxidation Positively Regulates Proofreading. To accurately decode mRNA, properly aminoacylated tRNA is required; otherwise, mistranslation can occur. During oxidative stress, there is an increase in the presence of noncognate Tyr in the amino acid pools, which poses a threat to PheRS fidelity (7). How PheRS responds to the increased presence of noncognate substrate during oxidative stress remained unknown. Mass spectrometry analysis and circular dichroism reveal that PheRS oxidation leads to a conformational change, producing a protein with less α-helices. It is not oxidation of a specific residue in PheRS, but rather a global effect on the protein's structure. These results are in contrast to the better characterized oxidation of a cysteine residue in ThrRS that alters proofreading activity (5). However, oxidation of proteins has been shown to generally impact the structure of proteins and encourage the formation of aggregates leading to altered cellular responses (15, 19, 22). Oxidative generation of an unstructured protein causes PheRS to function in a hyperaccurate manner by more rapidly hydrolyzing the ester linkage of noncognate Tyr-tRNA^{Phe} both in vitro and in vivo. Steady-state kinetic analyses reveal that the conformational change induced by oxidative stress does not impact cognate or noncognate amino acid activation or cognate aminoacylation activity to generate Phe-tRNA^{Phe}. These results reveal an instance in which oxidative stress

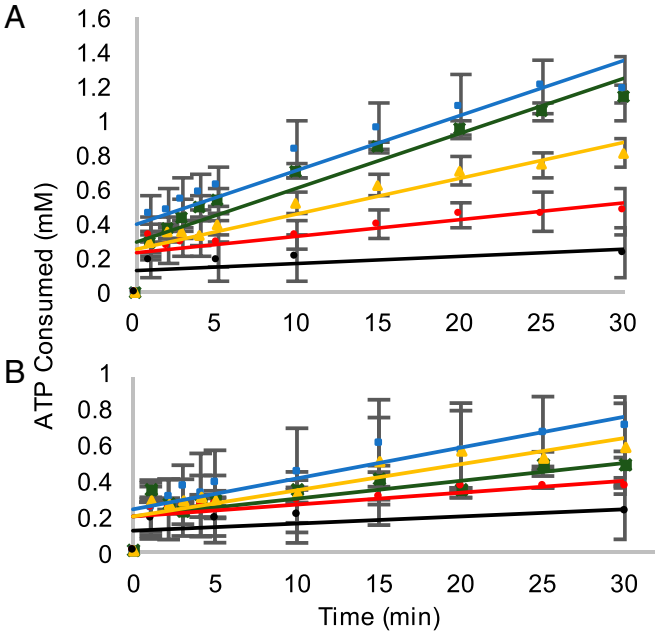

Fig. 4. ATP consumption using WT and editing-deficient PheRS. (*A*) ATP consumption with *m*-Tyr-tRNA^{Phe}. (*B*) ATP consumption with Phe-tRNA^{Phe}. WT PheRS (green), WT PheRS + 5 mM H_2O_2 (blue), editing-deficient PheRS (red), editing-deficient PheRS + 5 mM H_2O_2 (yellow), and no enzyme control (black).

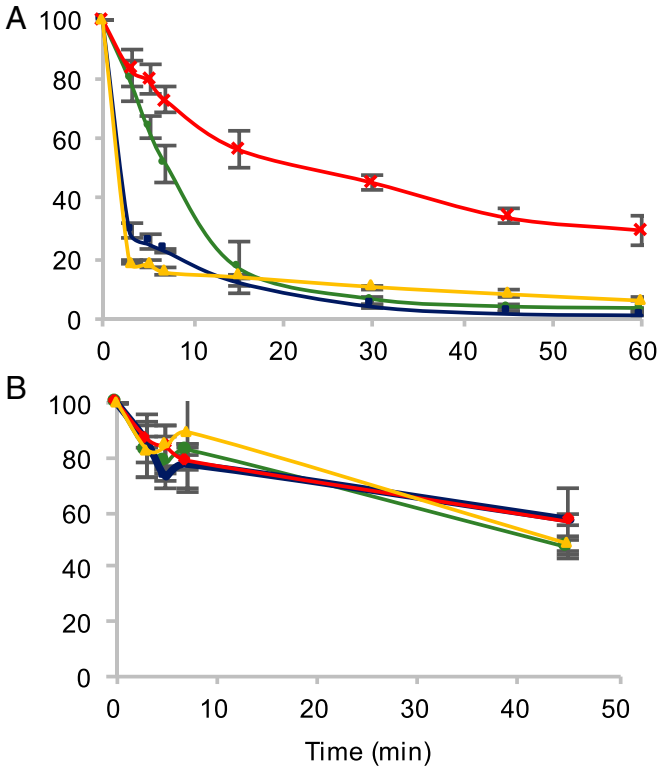

Fig. 5. Native *E. coli* PheRS is able to edit mischarged $tRNA^{Phe}$ using cell lysates grown under oxidative stress. (*A*) Hydrolysis of H^3 *p*-Tyr-$tRNA^{Phe}$ over time. (*B*) Hydrolysis of H^3 Phe-$tRNA^{Phe}$ over time. Editing-deficient PheRS (red), editing-deficient PheRS + 5 mM H_2O_2 (yellow), WT PheRS (green), WT PheRS + 5 mM H_2O_2 (blue).

positively regulates proofreading, whereas previous reports on the effect of oxidative stress on aaRS demonstrated that proofreading activity can be inhibited by H_2O_2 treatment (14). This highlights the importance of using an editing-deficient PheRS in our experiments because it allows us to notice changes in proofreading activity that may be more difficult to observe for WT protein (e.g., *SI Appendix*, Fig. S4). Furthermore, the lack of commercially available radiolabeled *m*-Tyr, the preferred noncognate substrate, may also contribute to the lack of observable statistically significant differences upon oxidation of WT PheRS in some assays (e.g., *SI Appendix*, Table S6).

Proofreading Protects Cells from Mistranslation Under Adverse Growth Conditions. Positively regulating proofreading provides one mechanism by which *S.* Typhimurium is able to maintain translational accuracy during oxidative stress. In addition, due to shifts in amino acid pools during oxidative stress generating elevated levels of noncognate substrates, the increased proofreading activity of PheRS may be important for maintaining cellular homeostasis. During oxygen-limiting growth, norvaline (Nva) accumulates and poses a threat to the accuracy of leucyl-tRNA synthetase (LeuRS) (23, 24). LeuRS uses posttransfer editing to hydrolyze Nva-$tRNA^{Leu}$ and prevent its incorporation into growing peptide chains. Similarly, during oxidative stress, Tyr isomers accumulate, posing a threat to the accuracy of PheRS. We have demonstrated that oxidation of PheRS increases translational quality control to help maintain high fidelity of aminoacylation upon exposure to high levels of noncognate Tyr isomers. Hyperaccuracy may be especially important to PheRS because the substrate for PheRS, Phe, can directly be oxidized to cytotoxic nonproteinogenic *m*-Tyr. It has also been demonstrated that oxidative stress effects aaRS proofreading adversely; however, in those cases, there is not a direct correlation to higher levels of noncognate amino acid in the cell. In *Candida albicans*, it has been reported that mistranslation may be beneficial by generating proteome diversity (25). In addition, phosphorylated MetRS is able to recognize diverse tRNAs in the cell to increase misincorporation of Met into the proteome (16). It is proposed that mismethionylation, leading to mistranslation, may be beneficial for the cell by allowing Met to quench oxygen radicals. Here we have reported that in *S.* Typhimurium, mistranslation may not be beneficial during oxidative stress due to the increase in presence of *o*-Tyr, *p*-Tyr, and *m*-Tyr. Overall, ensuring the accuracy of translation dependent on PheRS, rather than increasing proteome diversity, may be more important for the cell to survive during oxidative stress. Similarly, it has been proposed that the loss of proofreading upon oxidation of ThrRS may primarily function in detecting oxidant levels in the environment, rather than promoting potentially beneficial mistranslation (26).

Hyperaccuracy of Oxidized PheRS and the Stringent Response. The stringent response is a cellular stress response that is activated upon amino acid starvation. Upon accumulation of uncharged tRNA, an alarmone, guanosine pentaphosphate or tetraphosphate [(p)ppGpp], is produced to modulate transcription of amino acid biogenesis genes and suppress transcription of genes involved in translation (27). The stringent response aims to generate a balance between translation and the nutrients available. Inhibiting PheRS quality control prevents appropriate activation of the stringent response due to the accumulation of mischarged $tRNA^{Phe}$ (28). Without the stringent response, bacteria cannot appropriately respond

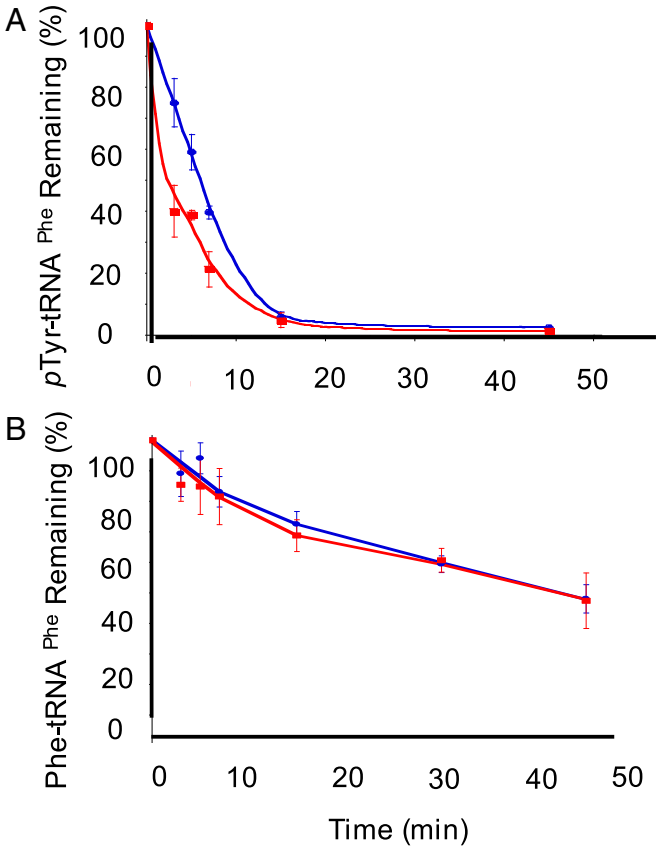

Fig. 6. *E. coli* cell lysates lacking scavenging enzymes (HPX^-) edit mischarged $tRNA^{Phe}$ better than WT *E. coli* grown anaerobically. (*A*) Hydrolysis of H^3 *p*-Tyr-$tRNA^{Phe}$ over time. (*B*) Hydrolysis of H^3 Phe-$tRNA^{Phe}$ over time. WT *E. coli* (blue) and HPX^- *E. coli* (red).

to nutrient-limiting environments. In *Salmonella*, regulation of the stringent response is required for many cellular properties such as growth and motility, as well as *Salmonella* host cell invasion (29). Therefore, increasing the accuracy of translation could allow for *S*. Typhimurium to maintain its ability to properly activate the stringent response and invade a host when amino acid pools are perturbed. Proper activation of stress responses allows for *S*. Typhimurium to colonize within and infect a host. Through this work, we have expanded the knowledge of the effects oxidative stress has on translational quality control in *S*. Typhimurium by defining hyperaccurate PheRS. *S*. Typhimurium can directly benefit from hyperaccuracy of translation through accurate protein production and proteome integrity and indirectly through proper activation of the stringent response during adverse growth conditions. *S*. Typhimurium can use these translational stress responses to complement the well-characterized transcriptional responses that are the foundation for *S*. Typhimurium pathogenesis to maintain resiliency during oxidative stress and survive within the host microbiome (30–32).

Methods

***Salmonella* PheRS Cloning and Purification.** Genomic DNA from *S*. Typhimurium 14028s (from John Gunn, The Ohio State University, Columbus, OH) was used to PCR-amplify *pheS* and *pheT*, which were cloned into pET28a(+). Editing-deficient *S*. Typhimurium PheRS was generated by site-directed mutagenesis in pheT (G318W). Plasmids containing proteins were expressed in *E. coli* BL21(DE3) with 1 mmol IPTG induction for 4 h. Cells were harvested, lysed by sonication, and purified using a TALON metal affinity resin. PheRS was eluted with 250 mM imidazole, and fractions containing protein were concentrated and dialyzed overnight in 50 mM Tris pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 3 mM 2-mercaptoethanol, 5% glycerol, and then dialyzed 4 h in similar buffer with 50% glycerol for storage.

Detection of Oxidation of PheRS. Purified protein was incubated with 0, 5, or 20 mM H_2O_2 at 37 °C for 1 h. Excess H_2O_2 was dialyzed out using a 3-kDa filtration unit. Fifteen micrograms of protein was incubated at 25 °C for 15 min, combined with equal-volume 12% SDS and 2× volume of either dinitrophenyl hydrazine (DNPH) or control solution. Neutralization buffer was added, and samples were run on a 10% SDS/PAGE gel. Gel was transferred onto a nitrocellulose membrane. Western blot was completed using antibody dilutions as recommended by the Millipore OxyBlot Protein Oxidation Detection Kit protocol. Image intensity was quantified using imageJ. Oxidation extent was normalized to a nonoxidized protein.

Enzymatic Assays. Detailed descriptions of methods used to analyze steady-state kinetics, proofreading of PheRS, and preparation of materials is described in the *SI Appendix, Supplemental Data Methods*.

Circular Dichroism. Purified proteins (0.2 mg/mL) in 100 mM KPO_4 were analyzed in a quartz absorption cuvette with 1 mm path length. Proteins were oxidized as in the MS preparation above. Absorbance values between 190 and 260 nm were recorded.

PheRS-Specific Activity of *E. coli* Cell Lysates. One hundred millimoles Na^{2+} Hepes (pH 7.2), 30 mM KCl, 10 mM $MgCl_2$, 2 mM ATP, 15 μM $tRNA^{Phe}$, 30 μM ^{14}C-Phe, and either 12 μg or 36 μg total protein from cell lysate were combined and incubated at 37 °C for 1 h. Time points were taken at 0 (before adding lysate), 5, 15, 30, 45, and 60 min and placed onto filter papers presoaked with 5% TCA. The filter papers were subsequently washed in 3× 5% TCA and 1× 95% EtOH. Radiation was quantified using liquid scintillation counting. The PheRS-specific activity was determined by [charged ^{14}C-Phe-$tRNA^{Phe}$ (pmol)/min]/total protein (mg).

Editing of p-Tyr-$tRNA^{Phe}$ Using *E. coli* Cell Lysates. *E. coli* strains were grown to an OD_{600} 0.4, and 5 mM H_2O_2 was added for 1 h. Cells were harvested, lysed by sonication, and lysate was cleared by centrifugation for 1 h at 22,000 *g* at 4 °C. Precharged pTyr-$tRNA^{Phe}$ was combined with 30 mM KCl, 10 mM $MgCl_2$, and 0.16 mg/mL of cell lysate, and reaction aliquots were taken between 0–60 min and placed onto 3-mm filter papers presoaked with 5% TCA. The filter papers were washed as above, and radiation was quantified using liquid scintillation counting.

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