Enhanced translation of leaderless mRNAs under oxidative stress in *Escherichia coli*

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15 **Abstract**

16 The bacterial response to oxidative stress requires the adaptation of the proteome to
 17 the hostile environment. It has been reported that oxidative stress induces a strong and global
 18 inhibition of both, transcription and translation. Nevertheless, whereas it is well known that
 19 transcription of a small group of genes is induced thanks to transcription factors such as
 20 OxyR and SoxR, an equivalent mechanism has not been described for translation. Here we
 21 report that whereas canonical translation that depends on Shine Dalgarno recognition is
 22 inhibited by oxidative stress in *Escherichia coli*, the translation of leaderless mRNA (lmRNA)
 23 is enhanced under such conditions. Both, inhibition of canonical translation and enhancement
 24 of lmRNA translation, depend on the production of (p)ppGpp. We propose that such a
 25 mechanism would allow bacteria to rapidly adapt their proteome to hostile conditions and is,
 26 perhaps, a general strategy to confront strong stressful conditions.

27 **Significance statement**

28 The regulation of translation (the production of proteins based on genetic information)
 29 is central for the adaptation to environmental changes. In *Escherichia coli* translation may
 30 begin through two alternative pathways. 1.- A canonical initiation that is well understood and
 31 is regulated mostly by changes in the accessibility of ribosomes to specific sequences and 2.-
 32 Initiation of leaderless mRNAs (lmRNAs) that lack these sequences and for which we do not
 33 understand the regulation process. Our results indicate that under oxidative stress, the
 34 production of (p)ppGpp in *E. coli* inhibits canonical translation and simultaneously enhances
 35 translation of lmRNAs, showing for the first time a natural condition where lmRNA translation
 36 is regulated and a role for (p)ppGpp in this process.

37 **Introduction**

38 Bacteria are exposed to continual environmental changes, where modification of the
 39 proteome allows adaptation to the new conditions. Regulation of bacterial translation is key to
 40 this process (Rothschild & Mancinelli, 2001) and usually depends on changes to the initiation
 41 step. This is usually the slowest step of translation, and thus, changes to it will produce the
 42 greatest effects on protein production (Gualerzi & Pon, 2015; Laursen et al., 2005). According
 43 to the canonical model, initiation of translation in bacteria starts with the positioning of the

small ribosomal subunit (30S) and initiator tRNA (fMet-tRNA) at the start codon in the mRNA, in conjunction with initiator factors 1 (IF1), 2 (IF2) and 3 (IF3), generating a pre-initiation complex (Gualerzi & Pon, 2015; Schmeing & Ramakrishnan, 2009). The region located upstream of the initiation codon, the leader region, guides the positioning of the start codon in the peptidyl-tRNA site (P-site) of the 30S subunit, by the interaction between the Shine Dalgarno (SD) sequence in the mRNA and a highly conserved segment of the 3' end of 16S rRNA called anti-SD (aSD) sequence. In addition, low folding stability (Saito et al., 2020) and interactions of the ribosomal protein S1 and enhancer sequences such as a A/U-rich region upstream of SD may aid binding and positioning of the mRNA to the small ribosomal subunit (Kaminishi et al., 2007; Komarova et al., 2002). Positioning of the 30S at the ribosome binding site (RBS) allows binding of the large ribosomal subunit (50S), hydrolysis of GTP by IF2 and the release of initiation factors. This enables the entry of the elongation factor Tu (EF-Tu) with aminoacylated tRNAs and the start of polypeptide synthesis (Gualerzi & Pon, 2015; Schmeing & Ramakrishnan, 2009). Given the relevance of the positioning of the 30S subunit on the RBS, any change of its accessibility will alter the speed of translation initiation. Thus, changes of RNA folding or binding of sRNA and proteins have been observed to be usually involved in translation regulation (Duval et al., 2015).

In addition to the canonical initiation of translation, bacteria may initiate translation from leaderless mRNA (lmRNA). These mRNA are characterized by its lack of the usual 5' UTR. They may present none or a small number of nucleotides and, most importantly, they completely lack any SD sequence. In contrast to the canonical initiation of bacterial translation, lmRNA translation has been proposed to start by binding of 70S or smaller 61S particles, not 30S (Beck et al., 2016; O'Donnell & Janssen, 2002; Udagawa et al., 2004). These kind of mRNA without a canonical RBS have been found in phages (e.g., λ and P2), transposons (e.g., Tn1721), and several bacterial species (e.g., *Mycobacterium smegmatis* and *Streptomyces clavuligerus*) (Cortes et al., 2013; de Groot et al., 2014; Hwang et al., 2019; T. G. Nguyen et al., 2020; Resch et al., 1995). Although they are common in nature, studies targeted to describe transcription initiation sites have usually found only a small number of lmRNA in enterobacteria. For instance, only 20 to 30 lmRNA were found in *E. coli* K-12 and *Salmonella enterica* serovar Typhimurium SL1344 (Kröger et al., 2012; Mendoza-Vargas et al., 2009). Nevertheless, it has been reported in other enterobacteria that transcription from alternative promoters may allow the production of lmRNA under hostile

conditions. For example, an alternative promoter has been described for *virF*, a transcriptional factor that regulates virulence of *Shigella* spp. during mammalian cell infection. This alternative promoter generates a lmrRNA that allows the production of a functional fragment of the *virF* regulator (Di Martino et al., 2016), suggesting a possible involvement of lmrRNAs translation in the adaptation to host-pathogen conditions.

Translation under oxidative stress

An example of the hostile conditions commonly confronted by bacteria is oxidative stress. Bacteria are often exposed to various environmental and anthropogenic factors that favor the production of oxidative molecules, which in some conditions, can produce oxidative stress (Imlay, 2019; Kataria & Ruhal, 2014). One of such conditions is the interaction with human immune cells, such as macrophages and polymorphonuclear leukocytes (G. T. Nguyen et al., 2017; Schlauch, 2011). As a consequence, microorganisms have developed diverse strategies to overcome this stress. Whereas the transcriptional regulation of this response has been described in great detail (Chiang & Schellhorn, 2012; Imlay, 2013), the literature on changes in the translational machinery under oxidative stress is more limited. However, many authors have described a global inhibition of translation as a consequence of oxidative stress (Fasnacht & Polacek, 2021; Kojima et al., 2007; Nishiyama et al., 2004; Shenton et al., 2006; Zhong et al., 2015; M. Zhu & Dai, 2019). In addition to this global repression of translation, we and other authors have described important alterations in translation elongation. For instance, an increased error rate in the incorporation of some amino acids (Ling & Soll, 2010; Steiner et al., 2019; Wu et al., 2014) or decreased elongation rates during translation have been observed (Leiva et al., 2020; Zhong et al., 2015; M. Zhu & Dai, 2019). The relative contribution of each of these effects is not completely understood. Some groups have proposed that tRNA degradation limits translation elongation, determining the global translation efficiency (Zhong et al., 2015; M. Zhu & Dai, 2019). Nevertheless, this effect depends on the stressor concentration and is probably affected by other aspects, such as culture conditions and particular strains used. In effect, we have not observed this tRNA degradation using another strain of *E. coli* (K-12 MG1655). When using 250 μ M of paraquat to induce oxidative stress, the decreased translation seems to derive mainly from steps different from elongation, probably translation initiation (Leiva et al., 2020).

To better understand the impact of oxidative stress on translation, here, we have

investigated the impact of this stress on translation initiation. Our results show that while translation of canonical mRNA is inhibited under oxidative stress, that of lmrRNAs is strongly induced. Furthermore, the results strongly suggest that these changes depend in part on the production of (p)ppGpp, an alarmone known to modulate the transcription and translation machinery in response to hostile environmental conditions.

Results

Oxidative stress induces the formation of a ribosomal particle of lower sedimentation coefficient

In order to get some insights about the effects of paraquat on translation, we analyzed the sedimentation coefficient of ribosomes from *E. coli* cultured in M9r media in the presence of 250 μ M paraquat. This is a technique commonly used to analyze translation, as it indicates the proportion of ribosomes that are involved in more efficient (polysome fraction) and more inefficient (monosome/70S fraction) translation. Previous assays have shown that under the oxidative stress condition used in this experiment, bacterial replication and protein synthesis are greatly decreased (Leiva et al., 2020). Also, for this strain, the decreased translation produced by this lower concentration of paraquat seems to depend mainly on steps different from elongation (Leiva et al., 2020). To this end, a sedimentation analysis of the components of the translational machinery was carried by ultracentrifugation in sucrose gradients (10-40%) (Qin & Fredrick, 2013). As expected, free 30S, and 50S subunits, a majority peak corresponding to monosomes (70S) and a fraction of polysomes were observed when analyzing cells cultured under control conditions (Figure 1A) (Qin & Fredrick, 2013). These peaks were also observed in extracts from cultures stressed with 250 μ M paraquat for 30 minutes (Figure 1B). However, we observed the appearance of a particle of intermediate sedimentation coefficient between the 50S subunit and complete ribosome (70S) peaks, whose abundance varies over time (Figure 1C). By interpolation, we have estimated that this particle has a sedimentation coefficient of 60 ± 0.6 S, very similar to particles observed after addition of Kasugamycin to *E. coli* cultures, especially when lmrRNAs are overexpressed. This antibiotic has been proposed to promote the loss of at least 6 ribosomal proteins of the minor subunit (some of them highly relevant for translation, such as S1 and S12) and induce the translation of lmrRNA (Delvillani et al., 2011; Kaberdina et al., 2009). Given the relevance that activation of translation of the lmrRNA subset of the transcriptome would have in the

adaptation to stressful conditions, we decided to further investigate the possibility that translation of lmrRNA is induced by paraquat dependent oxidative stress.

Translation of leaderless mRNA is enhanced by oxidative stress

Canonical and lmrRNA initiation are the only two mechanisms known to be capable of initiating translation of the first cistron of bacterial transcripts. To compare the efficiency of translation by both mechanisms under control and oxidative stress conditions, we used two genetic reporter plasmids that code for a bicistronic operon coding for two fluorescent proteins, GFP upstream of mCherry (Figure 2A). One of such plasmids, plasmid pSD, was used to study canonical initiation. In pSD, both reporter genes (*gfp* and *mCherry*) have a canonical RBS. A second plasmid, plmrRNA, was used to study lmrRNA initiation. In plmrRNA the first cistron (*gfp*) lacks a leader region, but the second cistron (mCherry) preserves a canonical RBS. Transcription initiation sites for both reporter plasmids were confirmed by reverse transcription coupled to template exchange (Langevin et al., 2013; Y. Y. Zhu et al., 2001). These experiments indicated that the lmrRNA transcript presents 3 nucleotides (ATA) upstream of the AUG initiation codon confirming its leaderless nature (Supplementary Table S1). Thus, in both plasmids mCherry initiation operates through the same canonical mechanism, but while initiation of *gfp* translation depends on canonical mechanisms for pSD, in plmrRNA *gfp* translation may only start through leaderless translation. As both genes, *gfp* and *mCherry*, are coded in the same RNA, alterations in the ratio of fluorescence measured from both proteins (GFP/mCherry) will reflect relative changes in the efficiency of both translation mechanisms.

When comparing fluorescence intensity kinetics of both reporters, we observed that translational levels of GFP and mCherry encoded in pSD decrease drastically under oxidative stress (Figure 2BC). In contrast, synthesis of GFP encoded in the leaderless transcript increased under oxidative stress (Figure 2B), while the levels of mCherry (that remains being translated by the canonical mechanism) show a small decrease (Figure 2C). Together, these results indicate that a strong inhibition of canonical initiation of translation occurs under oxidative stress, while efficiency of the translation of lmrRNA increases with respect to control condition. As a result, we concluded that *gfp* in an lmrRNA context is translated more efficiently than in a canonical context under oxidative stress. This interpretation derives from comparing the ratio between the speed of GFP and mCherry production (Figure 2E). As a plot

of the ratio between the values of two straight lines gives an asymptote that tends to the ratio of the slopes of both lines, a similar trend was observed by analyzing the ratio between the levels of GFP and mCherry after 2 hrs of induction (Figure 2DF) or a ratio between the areas under the curves in the linear region of slope ~ 0 of a GFP/mCherry vs time plot (Figure 2DG). All these approaches indicated an estimated 10 to 20 fold increase for the efficiency of translation of leaderless reporter with respect to the canonical reporter under oxidative stress induced by 250 μ M paraquat. pSD reporter does not show relevant changes of the GFP/mCherry ratio because translation efficiency is altered to a similar extent for both *gfp* and *mCherry* when it depends on canonical initiation. As the three methods give similar conclusions, further results are only expressed as the ratio of fluorescence at 2 hrs. To confirm this result does not derive from partial degradation of the reporter mRNAs, qRT-PCR was performed with probes targeted to 3 regions of the bicistronic reporter. As shown in supplementary figure S1, there are small changes on the levels of each region as a consequence of oxidative stress. Under oxidative stress, these changes of mRNA levels would produce a small increase of *gfp* mRNA for the SD reporter and a small increase of *mCherry* mRNA for the ImRNA reporter. Thus, changes of GFP/mCherry fluorescence does not derive from partial mRNA degradation. If anything, this result suggests that activation of ImRNA translation is stronger than interpreted exclusively from fluorescence changes. To further confirm that the GFP/mCherry method is useful to study alterations in ImRNA translation, we repeated this experiment inducing stress with kasugamycin, an antibiotic that is known to induce ImRNA translation (Kaberina et al., 2009; Schlutzen et al., 2006; Schuwirth et al., 2006). Kasugamycin induced a similar increase in the GFP/mCherry fluorescence ratio for the plmRNA reporter (Supplementary Figure S2) as observed with paraquat (Figure 2), further supporting our conclusion. Thus, *in toto*, our results strongly suggest that 250 μ M paraquat induce the translation of ImRNAs. To our knowledge, this is the first report of increased ImRNA translation in conditions that do not derive from antibiotics stress (Kaberina et al., 2009) or artificial deletion (Landwehr et al., 2021) or overexpression of genes (Vesper et al., 2011).

MazEF toxin and antitoxin are not involved in the oxidative stress activation of ImRNA translation

Although it has been observed that ImRNAs have a higher affinity for whole ribosomes

(70S) than for the minor subunit (30S) (O'Donnell & Janssen, 2002), today there is no clarity on the mechanism responsible for initiation of their translation. The formation of specialized ribosomes, responsible for the preferential translation of mRNA under certain stress conditions has been proposed. This process would be mediated by the MazEF toxin-antitoxin system, which would be responsible for partially digesting the 3' end of the 16S rRNA of the minor ribosome subunit (30S) and/or complete ribosomes (70S). This mechanism would generate ribosomes lacking 43 nucleotides (70S^{Δ43}) that contain the aSD sequence, causing a reduction in the affinity for mRNAs with a leader region, but maintaining translation of leaderless transcripts (Temmel et al., 2016; Vesper et al., 2011).

To evaluate whether the translation changes observed for the plmRNA reporter under oxidative stress depend on the action of MazEF, a mutant of the *mazEF* operon was constructed by homologous recombination with PCR products (Datsenko & Wanner, 2000). From the analysis of the translational kinetics using wild type *E. coli* K-12 MG1655 (K12) and mutant *E. coli* K-12 MG1655 $\Delta mazEF::FRT$ ($\Delta mazEF$), we observed that deletion of the MazEF toxin-antitoxin system does not prevent the strong increase in translation of *gfp* from the plmRNA reporter under oxidative stress (Figure 3A).

To further verify whether MazF or another endoribonuclease are processing the 16S rRNA under oxidative stress induced by paraquat, the integrity of its 3' end was analyzed by real-time RT-PCR. To this end, primers that hybridize at both sides of the proposed ACA cleavage site and indicate the levels of the non-cleaved 16S rRNA were used (primers S7 to Y12 in Figure 3B) (Vesper et al., 2011). A second pair of primers (S7 and X15) hybridizing a region conserved in all 16S rRNA species independent of their processing were used as an internal control of 16S rRNA levels. When analyzing the wild type strain, no significant changes were observed in the relative levels of the complete 3' end of the 16S rRNA after induction of oxidative stress with 250 μ M of paraquat. This suggests the absence of digestion at the 3' end of the 16S rRNA under oxidative stress (Figure 3C). Confirming these results, we were unable to detect the expected 43 nucleotide fragment by Northern blot analysis using the same probes that have been previously reported (Vesper et al., 2011) (data not shown).

Although in contradiction to the MazF model for lmrRNA translation activation, these results coincide with reports from other researchers that have suggested that the MazF endoribonuclease would not generate 70S^{Δ43} ribosomes. Instead, these researchers found that activation of MazF non-selectively degrades immature rRNA and mRNA (Culviner &

Laub, 2018; Mets et al., 2017). In accordance with those reports, when analyzing the levels of the 16S rRNA precursor using a primer that hybridizes to a region of the immature rRNA (downstream of the mature 3' extreme, Figure 3B), we observed that the levels of 16S pre-rRNA decrease dramatically after the induction of oxidative stress (Figure 3D). All together, these results suggest that in accordance with what was published by Mets *et al.*, 2017 and Culviner & Laub, 2018, in *E. coli* K-12 MG1655, 70S^{Δ43} ribosomes are not generated under oxidative stress. The results are also congruent with a decreased abundance of ribosomal RNA precursors that may derive from either their degradation or decreased transcription.

Oxidative stress increases the translational efficiency of ImRNAs naturally found in *E. coli*

Our results strongly supports that translation of ImRNA is activated under oxidative stress (Figure 2) in a mechanism that probably involves some changes in the composition of the ribosomal particles (Figure 1), but is independent of the processing of the 16S rRNA 3' extreme (Figure 3). Nevertheless, all these analyzes depend on experiments carried out using the plasmid plmRNA, which generates an artificial leaderless mRNA encoding the heterologous genes *gfp* and *mCherry*. However, leaderless transcripts can also be found in nature. For example, as a product of alternative promoters in regulatory genes from phages λ and P2, transposon Tn1721, or *virF* in *Shigella* spp. (Cortes et al., 2013; Di Martino et al., 2016; Resch et al., 1995). The first approaches to understand the mechanism of leaderless translation were carried out with the *cI* gene of phage λ. This gene has two described promoters, P_{RE} (promoter for repressor establishment) and P_{RM} (promoter for repressor maintenance). Transcription from P_{RE} generates a transcript with a 5' untranslated region of 403 nucleotides, while transcription beginning at P_{RM} produces a leaderless mRNA, whose 5' end corresponds to the AUG start codon (Schmeissner et al., 1980; Walz et al., 1976). To evaluate changes in the translation efficiency of the leaderless *cI* under oxidative stress, a translation fusion of the first 100 *cI* codons (from P_{RM}) to *gfp* was constructed in the vector plmRNA, forming reporter *cI-gfp* (Figure 4A). As expected, we observed an increase in GFP/mCherry ratio under oxidative stress (Figure 4C). Although activation of ImRNA translation is clear, this enhancement is more discreet than what we have observed using the plmRNA reporter (Figure 2). We are currently unable to explain this difference, but it is possible that it derives from differences between the sequence of the first codons of both

reporters of lmrRNA translation, as previous reports have shown idiosyncratic effects of diverse lmrRNA reporter sequences (Beck et al., 2016; Shell et al., 2015; Udagawa et al., 2004).

In addition to the synthesis of lmrRNA by transcription from promoters located near to the initiation codon, some authors have proposed that lmrRNAs may be also generated through cleavage by MazF of canonical mRNAs (Vesper et al., 2011). Possible MazF targets with their respective ACA cleavage sites have been described in *E. coli* strains that overexpress *mazF* (Sauert et al., 2016). Four of these genes belong to the SoxRS and OxyR regulons and thus, are potentially involved in the response to oxidative stress by paraquat: *fpr*, *katG*, *nepl* and *zwf* (Seo et al., 2015). Since the transcription initiation site of *katG* has been described (Tartaglia et al., 1989), this gene was chosen to study whether MazF may be involved in the production of lmrRNAs under oxidative stress. To this end, a translation fusion between the 5' extreme of *katG* (including the 5' UTR and the first 100 codons of the gene) and the *gfp* reporter was constructed. Furthermore, a point mutation was generated in the ACA cleavage site replacing it with AGA, to obtain a transcript that is not processable by MazF (Figure 4B). When analyzing *gfp* translation in the wild type strain, we observed no differences between the *katG*(ACA)-*gfp* and *katG*(AGA)-*gfp* reporters under control conditions (Figure 4D). However, under oxidative stress induced by 250 μ M of paraquat, we observed a higher GFP/mCherry ratio for the reporter that can be processed by MazF (ACA) (Figure 4D). When repeating these experiments in a strain lacking the toxin-antitoxin system, we did not observe any differences between the reporters, both under control and oxidative stress conditions (Figure 4D). Since we previously observed that MazF does not play a role in the increased translation of lmrRNA under oxidative stress (Figure 3A), these latest data suggest that MazF would participate in the processing of the *katG* transcript, increasing its translational efficiency under oxidative stress. As reporters differ only in the cleavage site described for MazF, it can be proposed that the increased translation of *katG*(ACA)-*gfp* is a consequence of the more efficient translation of at least a fraction of the mRNA that is processed into lmrRNA. This result is similar to that recently described for the *grcA* mRNA (MazF target), whose translation increases in a part of the bacterial population when MazF is overexpressed (Nikolic et al., 2017). Nevertheless, care should be taken in considering that induction of lmrRNA translation under stress conditions in the WT strain is modest, an effect that may derive from further digestion of transcripts cleaved by MazF (Culviner & Laub, 2018).

(p)ppGpp accumulation inhibits translation of canonical mRNAs under oxidative stress

Our results indicate that under stress conditions, a decrease in ribosomal RNA precursors is observed (Figure 3D). Such decrease may be a consequence of the accumulation of guanosine tetra- or penta-phosphate ((p)ppGpp) (Sanchez-Vazquez et al., 2019). This is a nucleotide alarmone produced when *E. coli* or other bacteria are exposed to decreased amino acids availability or several other conditions, including oxidative stress (Chang et al., 2002). It has been determined that this molecule is capable of interacting with around 30 proteins involved in different processes, including some involved in ribosome biogenesis and translation. As a consequence, (p)ppGpp inhibits transcription and translation of several genes (Zhang et al., 2018). Although it is well known that transcription of some genes is enhanced by (p)ppGpp (Sanchez-Vazquez et al., 2019), we are unaware of any report regarding its effects on l-mRNA translation. To determine whether this metabolite plays a role in the regulation of leaderless translation, we analyzed the efficiency of GFP production from pSD and plmRNA reporters in a mutant lacking the *relA* and *spoT* genes ($\Delta relA::FRT \Delta spoT::FRT$ or $\Delta relA \Delta spoT$), responsible for the synthesis of (p)ppGpp in *E. coli* (46). As mutants that cannot produce (p)ppGpp are unable to replicate in culture media devoid of most amino acids (Potrykus et al., 2011), we initially tried to perform the experiments in M9 media supplemented with triptone, a casein digest that contains all amino acids. Unexpectedly, in this culture medium we did not observe l-mRNA translation in either the WT or the $\Delta relA \Delta spoT$ strains, both under control or oxidative stress conditions (Data not shown). To overcome this limitation, WT and $\Delta relA \Delta spoT$ strains were cultured in M9 media supplemented with tryptone. Before performing the experiment, cultures were cleaned in M9 media and resuspended in M9 media with branched amino acids where they were used to test the production of GFP and mCherry. Using this protocol, we observed that l-mRNA translation is activated in the WT strain under oxidative stress, replicating our previous observations. Nevertheless, the $\Delta relA \Delta spoT$ strain was unable to translate the l-mRNA *gfp* reporter under any conditions. If we instead resuspended the cell in M9 media containing tryptone, the l-mRNA reporter was not translated in any of the conditions or strains (Figure 5). Interestingly, deletion of the genes involved in (p)ppGpp synthesis also prevented the inhibition of canonical GFP and mCherry translation under oxidative stress (Figure 5). Altogether, these results show that l-mRNA cannot be translated in strains impeded to produce sufficient levels of (p)ppGpp either due to

mutation of *relA* and *spoT* or because of a high amino acids availability. Thus, our work strongly suggests that in addition to inhibiting canonical translation, (p)ppGpp is required for the activation of ImRNA translation at least under oxidative stress. Deletion of only *relA* was not enough to prevent ImRNA translation under oxidative stress, although it did affect translation of canonical mRNAs (Supplementary Figure S3).

Part of the (p)ppGpp response is mediated by Lon protease activation that leads to degradation of ribosomal proteins (Kuroda, 2001). However, *lon* deletion does not prevent *gfp* expression from the ImRNA reporter (Supplementary Figure S3) indicating that ImRNA translation does not require the Lon dependent degradation of ribosomal proteins.

Discussion

Because initiation is usually the slowest step of translation, regulation of mRNA translation usually depends on alterations of this step. This is usually achieved by changes to the accessibility of the RBS, ultimately controlling binding of the 30S subunit (Duval et al., 2015). In addition to this regulation of translation of particular genes, global inhibition of translation has been observed as a response to several hostile conditions such as the stationary phase of bacterial cultures (Kato et al., 2010), when Mg^{+2} is limiting (Pontes et al., 2016) or as a response to cold shock (Vila-Sanjurjo et al., 2004). This decrease in translation usually depends on the inhibition of ribosomes, but in some cases changes in the accessibility of the RBS also plays a role. One of the best described mechanisms for global inhibition of bacterial translation is the early response to cold shock in *E. coli*. As temperature decreases, mRNA folds, preventing access of the ribosome to correct translation initiation sites. Thus, translation must be globally repressed, preventing initiation at incorrect sites. As a response, most *E. coli* ribosomes enter a hibernation state induced by binding of YfiA protein to the P and A sites of complete ribosomes (70S). This YfiA-70S complex is unable to catalyze translation (Vila-Sanjurjo et al., 2004). Nevertheless, in order to confront the cold shock, it is not enough to block mis-translation of folded mRNA. Bacteria must additionally enhance translation of genes that may aid its ability to overcome the stress conditions. It has been observed that the reduced number of functional ribosomes selectively translate a minor subset of the available mRNAs that code for RNA chaperons and other proteins that allow a

355 better adaptation to cold conditions. All these mRNA fold under cold conditions to a structure
356 where the RBS and enhancer sequences remain exposed to binding by the 30S allowing
357 translation initiation (Vila-Sanjurjo et al., 2004). Thus, the early cold shock response is
358 characterized by a global inhibition of translation together with the induction of translation of a
359 subset of genes required for cold shock response.

360 The response to oxidative stress seems to follow a similar behavior to what has been
361 observed under cold shock, although mechanisms appear to be different. Several authors
362 have observed a global inhibition of translation (Kojima et al., 2007; Nishiyama et al., 2004;
363 Shenton et al., 2006; Zhong et al., 2015; M. Zhu & Dai, 2019). Some of them have indicated
364 that this inhibition would derive from massive tRNA degradation (Zhong et al., 2015; M. Zhu &
365 Dai, 2019). Nevertheless, it seems that this would be a strain dependent behavior, as strain
366 K-12 MG1655 used for this report does not show such massive decrease in tRNA content
367 (Leiva et al., 2020). Here we have observed that (p)ppGpp production may be responsible for
368 at least part of such inhibition. Perhaps, this may derive from the ability of (p)ppGpp to bind to
369 some translation factors using the GTP binding sites (Kanjee et al., 2012). Interestingly, this is
370 the case of IF2 (Mitkevich et al., 2010) that is required for canonical, but not for ImRNA
371 translation (Udagawa et al., 2004). Additional inhibition may derive from direct oxidation of
372 proteins and RNA involved in the translation process (Katz & Orellana, 2012; Liu et al., 2012;
373 Willi et al., 2018). Independent of the mechanism underlying global translation inhibition,
374 bacteria will need to preferentially translate genes that allow adaptation to oxidative
375 conditions. Otherwise, the effects of transcriptional control on bacterial adaptation would be
376 limited by a slow translation apparatus. Here we have observed that translation of ImRNA is
377 activated under oxidative stress conditions, apparently by the same (p)ppGpp that inhibits
378 canonical translation. This activation may be an indirect consequence of the increased
379 availability of ribosomes that show decreased translation of canonical mRNAs when
380 translation is inhibited by (p)ppGpp. Also, high levels of aminoacylated initiator tRNA, which
381 probably increase as a consequence of canonical translation inhibition, have been shown to
382 enhance ImRNA translation *in vitro* and could also be involved in the regulatory process
383 (Udagawa et al., 2004). Recently, the ATPase YchF of *E. coli* has been shown to bind the
384 ribosomes and apparently inhibit ImRNA translation (Landwehr et al., 2021). Thus, it is
385 possible that (p)ppGpp may inhibit YchF, although it is also possible that YchF somehow
386 prevents (p)ppGpp production or that it controls ImRNA translation by a (p)ppGpp

independent mechanism.

Understanding the mechanisms involved in (p)ppGpp activation of ImRNA translation will require further investigation. Nevertheless, the apparent ability of (p)ppGpp to simultaneously inhibit canonical translation and enhance ImRNA translation could allow for the selective translation of a subset of the transcriptome under oxidative stress. Additional experiments will be also required to determine this subset of ImRNAs, allowing a better understanding of the translational control for the adaptation to oxidative stress. If confirmed, these results suggest that bacteria may confront diverse hostile conditions through a common strategy of translation regulation, that is, a global inhibition of translation together with a concomitant activation of the translation of particular genes.

Materials and Methods

A brief description of the main methods used in this work is provided here. A detailed description can be found at the supplementary methods section.

Strains and culture media

All experiments performed in this work used wild-type (WT) *E. coli* K-12 MG1655 strain. Strains were cultured in either lysogeny broth (LB) media (1% tryptone, 0.5% yeast extract, and 0.5% NaCl), or M9 media (47.7 mM Na₂HPO₄, 22.0 mM KH₂PO₄, 8.6 mM NaCl, 18.7 mM NH₄Cl, 2 mM MgSO₄, 0.1 mM CaCl₂, and 0.4% Glycerol). When indicated, branched amino acids (isoleucine, leucine, and valine 50 µg/ml each), 0.1% tryptone, 0.4% arabinose 0.4%, ampicillin (100 µg/ml) or 250 µM paraquat were added to the culture media.

Analysis of polysomes

The detailed protocol for analysis of polysomes has been described by Qin and Kurt (2013). In brief, 50 ml of M9 media supplemented with branched amino acids were inoculated with saturated overnight culture in M9 media supplemented with tryptone and grown at 37°C in an orbital shaker. When indicated, the media also contained 250 µM paraquat. When bacteria reached mid-log phase (OD 600 ~0.4–0.6), cells were collected, lysed and prepared for ultracentrifugation as indicated in the supplementary methods. Samples were then loaded

on a 10%-40% sucrose gradient and centrifuged in a SW41 rotor at 35000 RPM for 3 hours. Finally, samples were analyzed using an in-line UV detector (ISCO/Brandel system).

Translation Efficiency Analyses

50 µl aliquot of mid-log phase cultures were diluted in a 96-well optical-bottom plate with 150 µl fresh M9 media supplemented with branched amino acids (50 µg/ml each) and arabinose (0.4% final concentration). When indicated, culture media contained paraquat (250 µM final concentration). Also 0.5% tryptone was used instead of branched amino acids in some experiments. Plates were further shaken at 37°C. OD 600 and fluorescence intensity of GFP (Ex. 480 ± 4.5 nm, Em. 515 ± 10 nm) and mCherry (Ex. 555 ± 4.5 nm, Em. 600 ± 10 nm) were measured in a microplate reader (Infinite M200 PRO, Tecan). In all experiments, strains transformed with pBAD30 (parental plasmid of pBAD30SFIT) were used to subtract the fluorescence background. A detailed description of the protocol can be found at the supplementary methods section.

Author contribution

The project was originally designed by MI, AK and OO. All experiments were performed by LL. AK, MI and OO supervised experiments and data interpretation. LL and AK wrote the paper with contributions from all authors. All authors reviewed and approved the final version of the manuscript.

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References

Beck, H. J., Fleming, I. M. C., & Janssen, G. R. (2016). 5'-Terminal AUGs in *Escherichia coli* mRNAs with Shine-Dalgarno Sequences: Identification and Analysis of Their Roles in

443 Non-Canonical Translation Initiation. *PLOS ONE*, 11(7), e0160144.
 444 <https://doi.org/10.1371/journal.pone.0160144>

445 Chang, D.-E., Smalley, D. J., & Conway, T. (2002). Gene expression profiling of
 446 *Escherichia coli* growth transitions: An expanded stringent response model. *Molecular*
 447 *Microbiology*, 45(2), 289–306. <https://doi.org/10.1046/j.1365-2958.2002.03001.x>

448 Chiang, S. M., & Schellhorn, H. E. (2012). Regulators of oxidative stress response genes in
 449 *Escherichia coli* and their functional conservation in bacteria. *Archives of Biochemistry and*
 450 *Biophysics*, 525(2), 161–169. <https://doi.org/10.1016/j.abb.2012.02.007>

451 Cortes, T., Schubert, O. T., Rose, G., Arnvig, K. B., Comas, I., Aebersold, R., & Young, D.
 452 B. (2013). Genome-wide Mapping of Transcriptional Start Sites Defines an Extensive
 453 Leaderless Transcriptome in *Mycobacterium tuberculosis*. *Cell Reports*, 5(4), 1121–1131.
 454 <https://doi.org/10.1016/j.celrep.2013.10.031>

455 Culviner, P. H., & Laub, M. T. (2018). Global Analysis of the *E. coli* Toxin MazF Reveals
 456 Widespread Cleavage of mRNA and the Inhibition of rRNA Maturation and Ribosome
 457 Biogenesis. *Molecular Cell*, 70(5), 868–880.e10. <https://doi.org/10.1016/j.molcel.2018.04.026>

458 Datsenko, K. A., & Wanner, B. L. (2000). One-step inactivation of chromosomal genes in
 459 *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences*,
 460 97(12), 6640–6645. <https://doi.org/10.1073/pnas.120163297>

461 de Groot, A., Roche, D., Fernandez, B., Ludanyi, M., Cruveiller, S., Pignol, D., Vallenet, D.,
 462 Armengaud, J., & Blanchard, L. (2014). RNA Sequencing and Proteogenomics Reveal the
 463 Importance of Leaderless mRNAs in the Radiation-Tolerant Bacterium *Deinococcus deserti*.
 464 *Genome Biology and Evolution*, 6(4), 932–948. <https://doi.org/10.1093/gbe/evu069>

465 Delvillani, F., Papiani, G., Dehò, G., & Briani, F. (2011). S1 ribosomal protein and the
 466 interplay between translation and mRNA decay. *Nucleic Acids Research*, 39(17), 7702–7715.
 467 <https://doi.org/10.1093/nar/gkr417>

468 Di Martino, M. L., Romilly, C., Wagner, E. G. H., Colonna, B., & Prosseda, G. (2016). One
 469 Gene and Two Proteins: A Leaderless mRNA Supports the Translation of a Shorter Form of
 470 the *Shigella* VirF Regulator. *MBio*, 7(6), e01860–16. <https://doi.org/10.1128/mBio.01860-16>

471 Duval, M., Simonetti, A., Caldelari, I., & Marzi, S. (2015). Multiple ways to regulate
 472 translation initiation in bacteria: Mechanisms, regulatory circuits, dynamics. *Biochimie*, 114,
 473 18–29. <https://doi.org/10.1016/j.biochi.2015.03.007>

474 Fasnacht, M., & Polacek, N. (2021). Oxidative Stress in Bacteria and the Central Dogma of
 475 Molecular Biology. *Frontiers in Molecular Biosciences*, 8, 671037.
 476 <https://doi.org/10.3389/fmolb.2021.671037>

477 Gualerzi, C. O., & Pon, C. L. (2015). Initiation of mRNA translation in bacteria: Structural
 478 and dynamic aspects. *Cellular and Molecular Life Sciences*, 72(22), 4341–4367.

479 <https://doi.org/10.1007/s00018-015-2010-3>

480 Hauryliuk, V., Atkinson, G. C., Murakami, K. S., Tenson, T., & Gerdes, K. (2015). Recent
481 functional insights into the role of (p)ppGpp in bacterial physiology. *Nature Reviews*
482 *Microbiology*, 13(5), 298–309. <https://doi.org/10.1038/nrmicro3448>

483 Hwang, S., Lee, N., Jeong, Y., Lee, Y., Kim, W., Cho, S., Palsson, B. O., & Cho, B.-K.
484 (2019). Primary transcriptome and translome analysis determines transcriptional and
485 translational regulatory elements encoded in the *Streptomyces clavuligerus* genome. *Nucleic*
486 *Acids Research*, 47(12), 6114–6129. <https://doi.org/10.1093/nar/gkz471>

487 Imlay, J. A. (2013). The molecular mechanisms and physiological consequences of
488 oxidative stress: Lessons from a model bacterium. *Nature Reviews Microbiology*, 11(7), 443–
489 454. <https://doi.org/10.1038/nrmicro3032>

490 Imlay, J. A. (2019). Where in the world do bacteria experience oxidative stress?: Oxidative
491 stress in natural environments. *Environmental Microbiology*, 21(2), 521–530.
492 <https://doi.org/10.1111/1462-2920.14445>

493 Kaberdina, A. C., Szaflarski, W., Nierhaus, K. H., & Moll, I. (2009). An Unexpected Type of
494 Ribosomes Induced by Kasugamycin: A Look into Ancestral Times of Protein Synthesis?
495 *Molecular Cell*, 33(2), 227–236. <https://doi.org/10.1016/j.molcel.2008.12.014>

496 Kaminishi, T., Wilson, D. N., Takemoto, C., Harms, J. M., Kawazoe, M., Schlutzen, F.,
497 Hanawa-Suetsugu, K., Shirouzu, M., Fucini, P., & Yokoyama, S. (2007). A Snapshot of the
498 30S Ribosomal Subunit Capturing mRNA via the Shine-Dalgarno Interaction. *Structure*, 15(3),
499 289–297. <https://doi.org/10.1016/j.str.2006.12.008>

500 Kanjee, U., Ogata, K., & Houry, W. A. (2012). Direct binding targets of the stringent
501 response alarmone (p)ppGpp: Protein targets of ppGpp. *Molecular Microbiology*, 85(6), 1029–
502 1043. <https://doi.org/10.1111/j.1365-2958.2012.08177.x>

503 Kataria, R., & Ruhal, R. (2014). Microbiological Metabolism Under Chemical Stress. In
504 *Microbial Biodegradation and Bioremediation* (pp. 497–509). Elsevier.
505 <https://doi.org/10.1016/B978-0-12-800021-2.00021-2>

506 Kato, T., Yoshida, H., Miyata, T., Maki, Y., Wada, A., & Namba, K. (2010). Structure of the
507 100S Ribosome in the Hibernation Stage Revealed by Electron Cryomicroscopy. *Structure*,
508 18(6), 719–724. <https://doi.org/10.1016/j.str.2010.02.017>

509 Katz, A., & Orellana, O. (2012). Protein Synthesis and the Stress Response. In M. Biyani
510 (Ed.), *Cell-Free Protein Synthesis*. InTech. <https://doi.org/10.5772/50311>

511 Kojima, K., Oshita, M., Nanjo, Y., Kasai, K., Tozawa, Y., Hayashi, H., & Nishiyama, Y.
512 (2007). Oxidation of elongation factor G inhibits the synthesis of the D1 protein of
513 photosystem II. *Molecular Microbiology*, 65(4), 936–947. <https://doi.org/10.1111/j.1365-2958.2007.05836.x>

515 Komarova, A. V., Tchufistova, L. S., Supina, E. V., & Boni, I. V. (2002). Protein S1
516 counteracts the inhibitory effect of the extended Shine-Dalgarno sequence on translation.
517 *RNA*, 8(9), 1137–1147. <https://doi.org/10.1017/S1355838202029990>

518 Kröger, C., Dillon, S. C., Cameron, A. D. S., Papenfort, K., Sivasankaran, S. K., Hokamp,
519 K., Chao, Y., Sittka, A., Hébrard, M., Händler, K., Colgan, A., Leekitcharoenphon, P.,
520 Langridge, G. C., Lohan, A. J., Loftus, B., Lucchini, S., Ussery, D. W., Dorman, C. J.,
521 Thomson, N. R., ... Hinton, J. C. D. (2012). The transcriptional landscape and small RNAs of
522 *Salmonella enterica* serovar Typhimurium. *Proceedings of the National Academy of Sciences*,
523 109(20), E1277–E1286. <https://doi.org/10.1073/pnas.1201061109>

524 Kuroda, A. (2001). Role of Inorganic Polyphosphate in Promoting Ribosomal Protein
525 Degradation by the Lon Protease in *E. coli*. *Science*, 293(5530), 705–708.
526 <https://doi.org/10.1126/science.1061315>

527 Landwehr, V., Milanov, M., Angebauer, L., Hong, J., Jüngert, G., Hiersemenzel, A., Siebler,
528 A., Schmit, F., Öztürk, Y., Dannenmaier, S., Drepper, F., Warscheid, B., & Koch, H.-G.
529 (2021). The Universally Conserved ATPase YchF Regulates Translation of Leaderless mRNA
530 in Response to Stress Conditions. *Frontiers in Molecular Biosciences*, 8, 643696.
531 <https://doi.org/10.3389/fmolb.2021.643696>

532 Langevin, S. A., Bent, Z. W., Solberg, O. D., Curtis, D. J., Lane, P. D., Williams, K. P.,
533 Schoeniger, J. S., Sinha, A., Lane, T. W., & Branda, S. S. (2013). Peregrine: A rapid and
534 unbiased method to produce strand-specific RNA-Seq libraries from small quantities of
535 starting material. *RNA Biology*, 10(4), 502–515. <https://doi.org/10.4161/rna.24284>

536 Laursen, B. S., Sørensen, H. P., Mortensen, K. K., & Sperling-Petersen, H. U. (2005).
537 Initiation of Protein Synthesis in Bacteria. *Microbiology and Molecular Biology Reviews*, 69(1),
538 101–123. <https://doi.org/10.1128/MMBR.69.1.101-123.2005>

539 Leiva, L. E., Pincheira, A., Elgamal, S., Kienast, S. D., Bravo, V., Leufken, J., Gutiérrez, D.,
540 Leidel, S. A., Ibba, M., & Katz, A. (2020). Modulation of *Escherichia coli* Translation by the
541 Specific Inactivation of tRNA^{Gly} Under Oxidative Stress. *Frontiers in Genetics*, 11, 856.
542 <https://doi.org/10.3389/fgene.2020.00856>

543 Ling, J., & Soll, D. (2010). Severe oxidative stress induces protein mistranslation through
544 impairment of an aminoacyl-tRNA synthetase editing site. *Proceedings of the National*
545 *Academy of Sciences*, 107(9), 4028–4033. <https://doi.org/10.1073/pnas.1000315107>

546 Liu, M., Gong, X., Alluri, R. K., Wu, J., Sablo, T., & Li, Z. (2012). Characterization of RNA
547 damage under oxidative stress in *Escherichia coli*. *Biological Chemistry*, 393(3), 123–132.
548 <https://doi.org/10.1515/hsz-2011-0247>

549 Mendoza-Vargas, A., Olvera, L., Olvera, M., Grande, R., Vega-Alvarado, L., Taboada, B.,
550 Jimenez-Jacinto, V., Salgado, H., Juárez, K., Contreras-Moreira, B., Huerta, A. M., Collado-
551 Vides, J., & Morett, E. (2009). Genome-Wide Identification of Transcription Start Sites,

552 Promoters and Transcription Factor Binding Sites in *E. coli*. *PLoS ONE*, 4(10), e7526.
553 <https://doi.org/10.1371/journal.pone.0007526>

554 Mets, T., Lippus, M., Schryer, D., Liiv, A., Kasari, V., Paier, A., Maiväli, Ü., Remme, J.,
555 Tenson, T., & Kaldalu, N. (2017). Toxins MazF and MqsR cleave *Escherichia coli* rRNA
556 precursors at multiple sites. *RNA Biology*, 14(1), 124–135.
557 <https://doi.org/10.1080/15476286.2016.1259784>

558 Mitkevich, V. A., Ermakov, A., Kulikova, A. A., Tankov, S., Shyp, V., Soosaar, A., Tenson,
559 T., Makarov, A. A., Ehrenberg, M., & Haurlyiuk, V. (2010). Thermodynamic Characterization
560 of ppGpp Binding to EF-G or IF2 and of Initiator tRNA Binding to Free IF2 in the Presence of
561 GDP, GTP, or ppGpp. *Journal of Molecular Biology*, 402(5), 838–846.
562 <https://doi.org/10.1016/j.jmb.2010.08.016>

563 Nguyen, G. T., Green, E. R., & Mecsas, J. (2017). Neutrophils to the ROScues:
564 Mechanisms of NADPH Oxidase Activation and Bacterial Resistance. *Frontiers in Cellular*
565 *and Infection Microbiology*, 7, 373. <https://doi.org/10.3389/fcimb.2017.00373>

566 Nguyen, T. G., Vargas-Blanco, D. A., Roberts, L. A., & Shell, S. S. (2020). The Impact of
567 Leadered and Leaderless Gene Structures on Translation Efficiency, Transcript Stability, and
568 Predicted Transcription Rates in *Mycobacterium smegmatis*. *Journal of Bacteriology*, 202(9),
569 e00746-19. <https://doi.org/10.1128/JB.00746-19>

570 Nikolic, N., Didara, Z., & Moll, I. (2017). MazF activation promotes translational
571 heterogeneity of the *grcA* mRNA in *Escherichia coli* populations. *PeerJ*, 5, e3830.
572 <https://doi.org/10.7717/peerj.3830>

573 Nishiyama, Y., Allakhverdiev, S. I., Yamamoto, H., Hayashi, H., & Murata, N. (2004).
574 Singlet Oxygen Inhibits the Repair of Photosystem II by Suppressing the Translation
575 Elongation of the D1 Protein in *Synechocystis* sp. PCC 6803. *Biochemistry*, 43(35), 11321–
576 11330. <https://doi.org/10.1021/bi036178q>

577 O'Donnell, S. M., & Janssen, G. R. (2002). Leaderless mRNAs Bind 70S Ribosomes More
578 Strongly than 30S Ribosomal Subunits in *Escherichia coli*. *Journal of Bacteriology*, 184(23),
579 6730–6733. <https://doi.org/10.1128/JB.184.23.6730-6733.2002>

580 Pontes, M. H., Yeom, J., & Groisman, E. A. (2016). Reducing Ribosome Biosynthesis
581 Promotes Translation during Low Mg²⁺ Stress. *Molecular Cell*, 64(3), 480–492.
582 <https://doi.org/10.1016/j.molcel.2016.05.008>

583 Potrykus, K., Murphy, H., Philippe, N., & Cashel, M. (2011). ppGpp is the major source of
584 growth rate control in *E. coli*: PpGpp and growth rate control. *Environmental Microbiology*,
585 13(3), 563–575. <https://doi.org/10.1111/j.1462-2920.2010.02357.x>

586 Qin, D., & Fredrick, K. (2013). Analysis of Polysomes from Bacteria. In *Methods in*
587 *Enzymology* (Vol. 530, pp. 159–172). Elsevier. <https://doi.org/10.1016/B978-0-12-420037->

588 1.00008-7

589 Resch, A., Tedin, K., Graschopf, A., Haggård-Ljungquist, E., & Bläsi, U. (1995). Ternary
590 complex formation on leaderless phage mRNA. *FEMS Microbiology Reviews*, 17(1–2), 151–
591 157. <https://doi.org/10.1111/j.1574-6976.1995.tb00197.x>

592 Rothschild, L. J., & Mancinelli, R. L. (2001). Life in extreme environments. *Nature*,
593 409(6823), 1092–1101. <https://doi.org/10.1038/35059215>

594 Saito, K., Green, R., & Buskirk, A. R. (2020). Translational initiation in *E. coli* occurs at the
595 correct sites genome-wide in the absence of mRNA-rRNA base-pairing. *ELife*, 9, e55002.
596 <https://doi.org/10.7554/eLife.55002>

597 Sanchez-Vazquez, P., Dewey, C. N., Kitten, N., Ross, W., & Gourse, R. L. (2019).
598 Genome-wide effects on *Escherichia coli* transcription from ppGpp binding to its two sites on
599 RNA polymerase. *Proceedings of the National Academy of Sciences*, 116(17), 8310–8319.
600 <https://doi.org/10.1073/pnas.1819682116>

601 Sauert, M., Wolfinger, M. T., Vesper, O., Müller, C., Byrgazov, K., & Moll, I. (2016). The
602 MazF-regulon: A toolbox for the post-transcriptional stress response in *Escherichia coli*.
603 *Nucleic Acids Research*, 44(14), 6660–6675. <https://doi.org/10.1093/nar/gkw115>

604 Schlutzenzen, F., Takemoto, C., Wilson, D. N., Kaminishi, T., Harms, J. M., Hanawa-
605 Suetsugu, K., Szaflarski, W., Kawazoe, M., Shirouzu, M., Shirouzo, M., Nierhaus, K. H.,
606 Yokoyama, S., & Fucini, P. (2006). The antibiotic kasugamycin mimics mRNA nucleotides to
607 destabilize tRNA binding and inhibit canonical translation initiation. *Nature Structural &*
608 *Molecular Biology*, 13(10), 871–878. <https://doi.org/10.1038/nsmb1145>

609 Schmeing, T. M., & Ramakrishnan, V. (2009). What recent ribosome structures have
610 revealed about the mechanism of translation. *Nature*, 461(7268), 1234–1242.
611 <https://doi.org/10.1038/nature08403>

612 Schmeissner, U., Court, D., Shimatake, H., & Rosenberg, M. (1980). Promoter for the
613 establishment of repressor synthesis in bacteriophage lambda. *Proceedings of the National*
614 *Academy of Sciences*, 77(6), 3191–3195. <https://doi.org/10.1073/pnas.77.6.3191>

615 Schuwirth, B. S., Day, J. M., Hau, C. W., Janssen, G. R., Dahlberg, A. E., Cate, J. H. D., &
616 Vila-Sanjurjo, A. (2006). Structural analysis of kasugamycin inhibition of translation. *Nature*
617 *Structural & Molecular Biology*, 13(10), 879–886. <https://doi.org/10.1038/nsmb1150>

618 Seo, S. W., Kim, D., Szubin, R., & Palsson, B. O. (2015). Genome-wide Reconstruction of
619 OxyR and SoxRS Transcriptional Regulatory Networks under Oxidative Stress in *Escherichia*
620 *coli* K-12 MG1655. *Cell Reports*, 12(8), 1289–1299.
621 <https://doi.org/10.1016/j.celrep.2015.07.043>

622 Shell, S. S., Wang, J., Lapierre, P., Mir, M., Chase, M. R., Pyle, M. M., Gawande, R.,
623 Ahmad, R., Sarracino, D. A., Ioerger, T. R., Fortune, S. M., Derbyshire, K. M., Wade, J. T., &

Gray, T. A. (2015). Leaderless Transcripts and Small Proteins Are Common Features of the Mycobacterial Translational Landscape. *PLOS Genetics*, 11(11), e1005641. <https://doi.org/10.1371/journal.pgen.1005641>

Shenton, D., Smirnova, J. B., Selley, J. N., Carroll, K., Hubbard, S. J., Pavitt, G. D., Ashe, M. P., & Grant, C. M. (2006). Global Translational Responses to Oxidative Stress Impact upon Multiple Levels of Protein Synthesis. *Journal of Biological Chemistry*, 281(39), 29011–29021. <https://doi.org/10.1074/jbc.M601545200>

Slauch, J. M. (2011). How does the oxidative burst of macrophages kill bacteria? Still an open question: How do phagocytic ROS kill bacteria? *Molecular Microbiology*, 80(3), 580–583. <https://doi.org/10.1111/j.1365-2958.2011.07612.x>

Steiner, R. E., Kyle, A. M., & Ibba, M. (2019). Oxidation of phenylalanyl-tRNA synthetase positively regulates translational quality control. *Proceedings of the National Academy of Sciences*, 116(20), 10058–10063. <https://doi.org/10.1073/pnas.1901634116>

Tartaglia, L. A., Storz, G., & Ames, B. N. (1989). Identification and molecular analysis of oxyR-regulated promoters important for the bacterial adaptation to oxidative stress. *Journal of Molecular Biology*, 210(4), 709–719. [https://doi.org/10.1016/0022-2836\(89\)90104-6](https://doi.org/10.1016/0022-2836(89)90104-6)

Temmel, H., Müller, C., Sauert, M., Vesper, O., Reiss, A., Popow, J., Martinez, J., & Moll, I. (2016). The RNA ligase RtcB reverses MazF-induced ribosome heterogeneity in *Escherichia coli*. *Nucleic Acids Research*, 45(8), 4708–4721. <https://doi.org/10.1093/nar/gkw1018>

Udagawa, T., Shimizu, Y., & Ueda, T. (2004). Evidence for the Translation Initiation of Leaderless mRNAs by the Intact 70 S Ribosome without Its Dissociation into Subunits in Eubacteria. *Journal of Biological Chemistry*, 279(10), 8539–8546. <https://doi.org/10.1074/jbc.M308784200>

Vesper, O., Amitai, S., Belitsky, M., Byrgazov, K., Kaberdina, A. C., Engelberg-Kulka, H., & Moll, I. (2011). Selective Translation of Leaderless mRNAs by Specialized Ribosomes Generated by MazF in *Escherichia coli*. *Cell*, 147(1), 147–157. <https://doi.org/10.1016/j.cell.2011.07.047>

Vila-Sanjurjo, A., Schuwirth, B.-S., Hau, C. W., & Cate, J. H. D. (2004). Structural basis for the control of translation initiation during stress. *Nature Structural & Molecular Biology*, 11(11), 1054–1059. <https://doi.org/10.1038/nsmb850>

Walz, A., Pirrotta, V., & Ineichen, K. (1976). λ repressor regulates the switch between PR and Prm promoters. *Nature*, 262(5570), 665–669. <https://doi.org/10.1038/262665a0>

Willi, J., Küpfer, P., Evéquoz, D., Fernandez, G., Katz, A., Leumann, C., & Polacek, N. (2018). Oxidative stress damages rRNA inside the ribosome and differentially affects the catalytic center. *Nucleic Acids Research*, 46(4), 1945–1957. <https://doi.org/10.1093/nar/gkx1308>

- 660 Wu, J., Fan, Y., & Ling, J. (2014). Mechanism of oxidant-induced mistranslation by
661 threonyl-tRNA synthetase. *Nucleic Acids Research*, 42(10), 6523–6531.
662 <https://doi.org/10.1093/nar/gku271>
- 663 Zhang, Y., Zborníková, E., Rejman, D., & Gerdes, K. (2018). Novel (p)ppGpp Binding and
664 Metabolizing Proteins of *Escherichia coli*. *MBio*, 9(2). <https://doi.org/10.1128/mBio.02188-17>
- 665 Zhong, J., Xiao, C., Gu, W., Du, G., Sun, X., He, Q.-Y., & Zhang, G. (2015). Transfer RNAs
666 Mediate the Rapid Adaptation of *Escherichia coli* to Oxidative Stress. *PLOS Genetics*, 11(6),
667 e1005302. <https://doi.org/10.1371/journal.pgen.1005302>
- 668 Zhu, M., & Dai, X. (2019). Maintenance of translational elongation rate underlies the
669 survival of *Escherichia coli* during oxidative stress. *Nucleic Acids Research*, 47(14), 7592–
670 7604. <https://doi.org/10.1093/nar/gkz467>
- 671 Zhu, Y. Y., Machleder, E. M., Chenchik, A., Li, R., & Siebert, P. D. (2001). Reverse
672 transcriptase template switching: A SMART approach for full-length cDNA library
673 construction. *BioTechniques*, 30(4), 892–897. <https://doi.org/10.2144/01304pf02>

674 **Figure 1. Oxidative stress induces the appearance of an undescribed peak between**
675 **50S and 70S.** Sedimentation profiles of *E. coli* extracts separated in 10 to 40% sucrose
676 gradients and detected at 254 nm. A) Samples collected from cultures at control conditions
677 and B) 30 min oxidative stress (Paraquat 250 μ M). C) Similar experiments performed after 30,
678 60, 120 and 180 min of oxidative stress induced by 250 μ M Paraquat. Lowercase letters
679 indicate 30S (a), 50S (b), new peak (c), 70S (d) and polysomes (e).

Figure 2. ImRNA is translated more efficiently under oxidative stress. A) Scheme of leader (pSD) or leaderless (plmRNA) reporters used in B, C, D, E, F and G. The bicistronic reporter system codes superfolder GFP (*gfp*) and mCherry (*mCherry*), downstream of the P_{BAD} promoter. B) and C) Kinetics of fluorescence normalized by OD₆₀₀ for GFP (B) and mCherry (C). D) Kinetic of the ratio of fluorescence intensities between GFP and mCherry (GFP/mCherry) for pSD and plmRNA in control (M9) and oxidative stress (250 μM Paraquat) conditions. E) Ratio between the slopes of the linear segment of the GFP/OD₆₀₀ (0.5-2.0h) and mCherry/OD₆₀₀ (1-2h) kinetics, under control and stress conditions. F) GFP/mCherry ratio at 2 h after transcription induction, under control and stress conditions. G) Area under the curve (AUC) of the graph in figure 2B between 2 and 4 h after transcription induction, under control and stress condition. Statistical analyses: unpaired two tailed t test with Welch's correction. ****p<0,0001, ***p<0,001, *p<0,05.

Figure 3. MazF and the cleavage of 16S 3' extreme are not required to induce translation under oxidative stress. A) GFP/mCherry fluorescence ratio for wild-type and $\Delta mazEF::FRT$ ($\Delta mazEF$) *E. coli* strains carrying pSD and plmRNA constructions at 2 h after transcription induction in control and stress condition. B) PCR strategy used to analyse the integrity of 16S rRNA 3' extreme. C) and D) Integrity analysis of 16S rRNA (C) and pre 16S rRNA (D) from wild-type *E. coli* under control condition and 30 or 120 min after oxidative stress induction. Statistical analyses: unpaired two-tailed t test for A. One-way ANOVA and Dunnett's multiple comparisons test for C and D. ****p<0,0001, **p<0,01, *p<0,05.

Figure 4. Oxidative stress increases the efficiency of translation of genes naturally encoded as leaderless transcripts. A) Scheme of the translational fusion of the first 100 codons of leaderless *cl* transcript (from P_{RM} promoter) to the *gfp-mCherry* reporter system. B) Scheme of the translational fusion between the 5'UTR and the first 100 codons of *katG* transcript to *gfp-mCherry* reporter system. *katG(ACA)-gfp* includes the wild-type 5'UTR while *katG(AGA)-gfp* includes a point mutation in sequence ACA, recognized by MazF. C) GFP/mCherry fluorescence ratio of *cl-gfp* fusion at 2 h after transcription induction, in control and stress conditions. D) GFP/mCherry fluorescence ratio of *katG-gfp* fusion at 2 h after transcription induction, in control and stress conditions in the WT and $\Delta mazEF$ strains. Statistical analyses: unpaired two-tailed t test with Welch's correction for C. Unpaired two-tailed t test for D. ***p<0,001, **p<0,01.

Figure 5. The induction of ImRNA translation under oxidative stress depends on the synthesis of (p)ppGpp and the availability of amino acids. GFP/mCherry, GFP/DO600 and mCherry/DO600 ratios for WT (K12; A, B and C) and $\Delta relA::FRT \Delta spoT::FRT$ ($\Delta relA\Delta spoT$; D, E and F) *E. coli* strains carrying the pSD and plmRNA constructs, at 2 h after transcription induction in control (light gray) and oxidative stress conditions (dark gray). The M9 culture medium was supplemented with low concentrations of branched amino acids (ILV 50 μg/mL each) or high concentration of all amino acids (Tryptone 0.5%). Note that experiments in this figure were performed with three independent clones of the $\Delta relA\Delta spoT$ strain, explaining in part the higher dispersion of data. ND= Not detected. Statistical analyses: unpaired two tailed t test with Welch's correction. ****p<0,0001, ***p<0,001, **p<0,01, *p<0,05.