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# Selective translation during stress in *Escherichia coli*

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## Abstract

The bacterial stress response, a strategy to cope with environmental changes, is generally known to operate on the transcriptional level. Here, we discuss a novel paradigm for stress adaptation at the post-transcriptional level, based on the recent discovery of a stress-induced modified form of the translation machinery in *Escherichia coli* that is generated by MazF, the toxin component of the toxin–antitoxin (TA) module *mazEF*. Under stress, the induced endoribonuclease MazF removes the 3′-terminal 43 nucleotides of the 16S rRNA of ribosomes and, concomitantly, the 5′-untranslated regions (UTRs) of specific transcripts. This elegant mechanism enables selective translation due to the complementary effect of MazF on ribosomes and mRNAs, and also represents the first example of functional ribosome heterogeneity based on rRNA alteration.

### Keywords

*Escherichia coli*; stress response; MazF; ribosome heterogeneity; translation

## The ribosome: a translation machinery with variable specificity

In nature, including in animal and human hosts, the conditions of bacterial life are generally stressful. Thus, bacteria must constantly cope with adverse agents and conditions such as antibiotics and perpetual alterations in temperature, osmolarity, pH, and nutrient levels. Bacteria have developed a gene expression program called the stress response in order to cope with such environmental changes. One general mechanism to react to stress is the alteration of the transcriptional program. Specific conditions induce the synthesis of alternative s factors that bind to the catalytic core of the RNA polymerase and define its specificity for certain promoters. Consequently, during stress, the transcriptional profile of the cell is altered [1–3]. In *E. coli*, the key s factor of the general stress response is RpoS ($\sigma^S$), which is responsible for transcription of distinct genes (known as the $\sigma^S$-regulon) during stationary phase or under general stress [4]. At elevated temperatures, the expression of heat shock genes is turned on by the heat shock σ factor ($\sigma^{32}$), which is stabilized at high temperature [5]. Finally, in response to extracytoplasmic stress, RpoE ($\sigma^{24}$) is generated and redirects the RNA polymerase to alternative promoters required under these conditions [6].

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Besides the transcriptional stress response, it is conceivable to imagine an adaptation of gene expression to given conditions at the translational level. However, due to its structural and functional complexity, the ribosome is traditionally viewed as highly conserved machinery with an invariable rRNA and protein complement that performs the concerted steps required for protein synthesis. This perception of invariance of the most intricate ribonucleoprotein complex in the cell has been perpetuated by the determination of high resolution crystal structures of the ribosome at the beginning of the millennium, which implies that bacterial ribosomes are homogeneous and constantly equipped with the same set of ribosomal proteins and rRNA molecules.

In contrast to this concept, a few lines of evidence indicate the formation of distinct ribosomal subpopulations when bacteria encounter adverse conditions [7–10]. Ribosome heterogeneity was first reported almost 40 years ago, when a growth-rate dependent variation in the protein composition of *E. coli* ribosomes was described [7,8]. However, the first evidence for functionally specialized ribosomes was the formation of protein-depleted ribosomes in *E. coli* upon treatment with the antibiotic kasugamycin [11]. These ribosomes have a reduced protein complement of the small 30S subunit and selectively translate leaderless mRNAs that lack a 5′-UTR and thus start directly with an AUG start codon [11]. Although the formation of these ribosomes is caused by rather artificial conditions, this study underscored the potential for the intrinsic regulatory capacity of the ribosome.

In addition to ribosome heterogeneity due to alterations in the protein complement, it is conceivable that variability within the rRNA could likewise contribute to the functional specificity of the translational apparatus. In bacteria, the presence of multiple *rrn* operons encoding rRNA, which slightly differ in sequence, favor this notion. Another support for this idea comes from the halophilic archaeon *Haloarcula marismortui*, in which ribosome stability can be adapted to high temperature by variations in the rRNA [9]. At elevated temperature, transcription of the *rrnC* operon is induced. This operon encodes rRNA containing an increased amount of CG base pairs, thereby resulting in the biogenesis of temperature-stable ribosomes. However, the formation of these alternative ribosomes is based on *de novo* ribosome biogenesis, and moreover, no functional specificity can be attributed to these rRNA variations.

Recently, a completely novel pathway for the alteration of protein synthesis has been observed, which is based on the formation of functionally distinct ribosomes [12]. By virtue of 16S rRNA processing under stress conditions, the translation machinery is rendered specific for leaderless mRNAs, which are likewise formed by the same mechanism. The key player responsible for the alterations of rRNA and mRNA is the endoribonuclease MazF, the toxic partner of the TA system *mazEF*, which is induced under stress.

## The TA module *mazEF*

TA systems consist of a pair of genes that encode two components: a stable toxin and an unstable antitoxin that interferes with the lethal activity of the toxin. Initially, such genetic systems for bacterial cell death were found mainly in *E. coli* on low-copy-number plasmids, where they are responsible for what is called the post-segregational killing effect. When a

bacterium loses such a plasmid (or another extrachromosomal element), the cell dies because the unstable antitoxin is degraded faster than the more stable toxin (reviewed in [13–17]). The cells can be thought of as 'addicted' to the short-lived product, because its *de novo* synthesis is essential for their survival. Therefore, the TA modules were also called 'addiction modules' because they maintain the stability in the host of the extrachromosomal elements on which they are borne.

In recent years, a great deal of attention has been focused on the abundance of TA gene modules in chromosomes of many bacteria [17–24], including pathogens such as *Staphylococcus aureus* [25], *Bacillus anthracis* [26], and *Mycobacterium tuberculosis*, which carries at least 88 putative TA modules [27]. In *E. coli*, eight different TA systems have been described [18,24,28–36]. Each of these modules consists of a pair of genes, usually cotranscribed as operons, in which the downstream gene encodes a stable toxin and the upstream gene encodes a labile antitoxin. Among these, one of the most studied is the chromosomal TA system *mazEF*, which was the first to be described as regulatable and responsible for bacterial programmed cell death [18,37]. *E. coli mazEF* encodes the labile antitoxin, MazE, and the stable toxin, MazF [18]. Both *mazE* and *mazF* are coexpressed and negatively autoregulated at the transcriptional level [38]. *E. coli mazEF* is triggered by various stressful conditions, such as treatment with antibiotics that affect transcription or translation [39], an increase in the concentration of the alarmone ppGpp upon severe amino acid starvation [18], or DNA damage [39–41]. In addition, mazEF-mediated cell death in *E. coli* is a population phenomenon requiring the presence of the linear pentapeptide NNWNN, which is a quorum sensing factor called the extracellular death factor (EDF) [42–44].

MazF is a sequence-specific endoribonuclease that preferentially cleaves single-stranded mRNAs at ACA sequences [45]. As previously reported [45,46], MazF induction causes inhibition of protein synthesis. However, we have subsequently shown that, surprisingly, this inhibition is not complete: although MazF inhibits the synthesis of most proteins (about 90%), it selectively enables the specific synthesis of about 10% of proteins [47]. Some of those proteins are required for the death of most of the population. However, MazF also enables the synthesis of proteins that permit the survival of a small subpopulation under the same stressful conditions that cause *mazEF*-mediated cell death for the majority of the population. The underlying molecular mechanism leading to the selective translation of a particular set of mRNAs upon MazF induction in *E. coli* has recently been elucidated [12].

## STM: the stress translation machinery

Besides the reported MazF activity in mRNA degradation [45], MazF also cleaves ACA sites at or closely upstream of the AUG start codon of specific mRNAs (Figure 1) [12], thereby generating particular short-leadered and leaderless mRNAs. Surprisingly, this study identified the 16S rRNA of the 30S ribosomal subunit as a novel target of the MazF endoribonuclease [12]. Specifically, the toxin cleaves at an ACA site in the 16S rRNA that is located 5′ of helix 45 (h45) between nucleotides A1499 and A1500. Thus, cleavage results in the loss of h45 and the anti-Shine and Dalgarno (aSD) sequence (Figure 2) [12], which is required for translation initiation at canonical ribosome binding sites [48,49]. Thus, the 3′-terminal truncation of the 16S rRNA generates a specialized STM that selectively translates

leaderless mRNAs formed by MazF [12]. This unexpected observation suggests a hitherto uncharacterized stress adaptation mechanism in bacteria, entailing a subtle modulation of gene expression based on ribosome heterogeneity and selective processing of a subset of mRNAs [12].

## Physiological benefits of the STM

In contrast to the alteration of the transcriptional program, the newly discovered STM provides an unexpected and novel paradigm for post-transcriptional stress response in bacteria. These data suggest that any stressful condition that prevents the expression of the chromosomally borne *mazEF* module and permits the toxin MazF to act freely will trigger translational reprogramming by heterogeneous ribosomes. Such conditions include severe amino acid starvation, and thereby over production of ppGpp [18], DNA damage [39–41], high temperatures, and oxidative stress [39]. From a physiological point of view, a pathway using the specialized translation machinery is beneficial for cells facing adverse conditions because it enables a fast and efficient response to a variety of stressful conditions. The functionally distinct stress-ribosomes are generated from pre-existing and translationally active 70S ribosomes by simple truncation of their 16S rRNA at the 3′-end in a process we designate ‘metabolic recycling’. This stress response neither depends on the energy-consuming steps of biogenesis of novel ribosomes, nor on the synthesis of ribosome-bound factors that mediate translation regulation [50]. Moreover, the corresponding removal of the 5′-UTR of specific mRNAs results in the synthesis of specific stress response proteins without the need for alternative transcription or synthesis of protein regulators. Thus, the endoribonuclease MazF, which was reported to act as a destructive enzyme [45], can in fact also act as a constructive element: in response to environmental changes, it rapidly generates a unique translation machinery that modulates the translation program by the generation of a novel ‘leaderless regulon’ that is translated by the stressribosomes [12]. This system generates a distinct class of ‘stress proteins’, some of which are involved in cell death and others in cell survival [47].

## STM is perfectly adapted to operate under stressful conditions

The translation system that is composed of the leaderless mRNA regulon and stress ribosomes is not only generated under adverse conditions but is also probably most suitable to operate under such conditions. It is intriguing that leaderless mRNAs are present in all six kingdoms of life [51]; in eukaryotic systems, translation of this class of mRNA takes place even in the absence of a 5′-terminal cap-structure [52,53]. Therefore, it is conceivable that such transcripts might represent remnants of ancestral mRNAs from before the separation of the phylogenetic domains [54]. In this light, the ability to translate leaderless mRNAs might be a conserved and very basic evolutionary function of a simple, most likely one-subunit, protoribosome that was present early in evolution. Such a protoribosome might have attached amino acids to the termini of RNA templates rather than at internal positions [55]. Hence, we hypothesize that reducing the translational apparatus to the basic machinery required for leaderless mRNA translation might contribute to the robustness of protein synthesis under unfavorable conditions and bypass the factor requirements of canonical mRNA translation. Our hypothesis is supported by the observations that the translation of

leaderless mRNAs can be accomplished in the absence of the essential ribosomal proteins S1 and S2 [56], and by ribosomes deficient in more than six ribosomal proteins [11]. Moreover, in contrast to translation of canonical mRNAs, where initiation factor 3 (IF3) is required for fidelity of start codon selection, IF3 is dispensable for translation of leaderless mRNAs [57]. Thus, it is conceivable that the STM is particularly resistant to possible alteration of the ribosome, such as protein depletion, which is potentially caused under stressful conditions.

The observation that leaderless mRNAs can likewise be translated by nondissociated 70S ribosomes [58] adds weight to the physiological benefit of the leaderless mRNA regulon and the stress-resistant subpopulation of ribosomes. The population of 70S monosomes has been shown to accumulate under unfavorable conditions, for example during starvation or temperature stress [59–61]. Hence, it seems that the STM might be mimicking the one-subunit protoribosome [58]. Collectively, these results imply that, even though the efficiency of leaderless mRNA translation is low, the formation of specific leaderless transcripts could be advantageous because it ensures continuous translation even under adverse conditions. This is consistent with the finding that MazF activation leads to the synthesis of a subclass of stress proteins that contribute to the survival of a small subpopulation of *E. coli* [47].

Interestingly, it seems that lysogenic phages might also experience a survival benefit from the STM machinery, by coevolving an adaption to the specificity of the STM. The CI repressor of the temperate *E. coli* phage λ, which is responsible for maintenance of the lysogenic state, is encoded by a leaderless mRNA [62]. The observation that the frequency of lysogeny is increased during conditions of slow bacterial growth [63] can thus be easily reconciled with the STM pathway: The generation of a sub-population of stress-ribosomes during slow growth or under adverse conditions would directly ensure continuous synthesis of the λCI repressor protein, which is required to favor the lysogenic decision. Hence, the bacterial and lysogenic phage populations would benefit from selective translation during adverse physiological conditions.

## Concluding remarks

Here, we have focused on the recent discovery of a novel translation machinery generated by MazF-induction under stressful conditions [12]. These findings show that: (i) MazF cleaves at ACA sites at or closely upstream to AUG start codons of specific mRNAs, thereby generating leaderless mRNAs belonging to a novel ‘leaderless mRNA regulon’; and (ii) MazF targets the 16S rRNA within the 30S ribosomal subunit at the decoding center, thereby removing 43 nucleotides from the 3′ terminus. As these 43 nucleotides include the aSD region, the deficient ribosomes, which we call stress-ribosomes, are selective for the translation of the generated leaderless mRNAs. Hence, under stressful conditions, the induction of MazF results in the formation of a leaderless mRNA regulon and stress ribosomes that coordinately produce a distinct pool of stress proteins [47]. We emphasize that this STM provides a novel paradigm for a bacterial stress response at the post-transcriptional level.

Of course, as happens for every newly discovered mechanism providing a novel paradigm, there are still crucial questions. Two important issues we currently address in our laboratories are: (i) whether ribosome heterogeneity occurs inside individual cells or is distributed among subpopulations of cells [12]; and (ii) the fate of the specialized ribosomes upon stress relief [12]. Considering the enormous amount of energy a cell must provide for ribosome biogenesis, a potential ribosome repair mechanism might exist that would enable the recovery from stress [12]. Several other outstanding questions are as follows. (i) Is the endoribonuclease MazF solely responsible for generating the specialized STM, or might additional *E. coli* endoribonucleases be involved? For example, in addition to *mazEF*, the *E. coli* chromosome also carries the TA gene module *chpBIK*, which is partially homologous to the module *mazEF* [29]. Although MazF cleaves single-stranded mRNAs at the 3′ or 5′ side of the first A in the sequence ACA [45], the toxin partner ChpBK is a less-specific endoribonuclease that cleaves at the 3′ or 5′ side of the first A in the sequences ACA, ACG, or ACU [64]. Thus, studying ChpBK might reveal even more leaderless mRNAs, which would point to an increased heterogeneity of the translation machinery under various stressful conditions. (ii) Does the nature of the stressful conditions that induce MazF and/or ChpBK determine whether they will generate deficient ribosomes and specific leaderless mRNAs? (iii) What is the nature of the entire leaderless mRNA regulon generated upon MazF or ChpBK induction? Is it a function of various stressful conditions? Furthermore, most kinds of bacteria carry either the TA module *mazEF* or similar TA systems [18–27], therefore, they might have STMs similar to the one in *E. coli* described here.

Finally, it was recently reported that, in contrast to AUG initiation codons, internal AUG codons are preceded by a stem-loop structure in the mRNA [65]. Therefore, we consider the possibility that the presence of ACA cleavage sites upstream of inframe AUG codons within coding regions could likewise increase the translation variability under stressful conditions through use of the STM. Hence, one could envisage that the prokaryotic STM, by its ability to form shorter leaderless mRNAs encoding shorter proteins, might be functionally analogous to the RNA splicing system in eukaryotes. Like the RNA splicing machineries, the STM could have the potential to increase the repertoire of the genomic information under specific physiological conditions.

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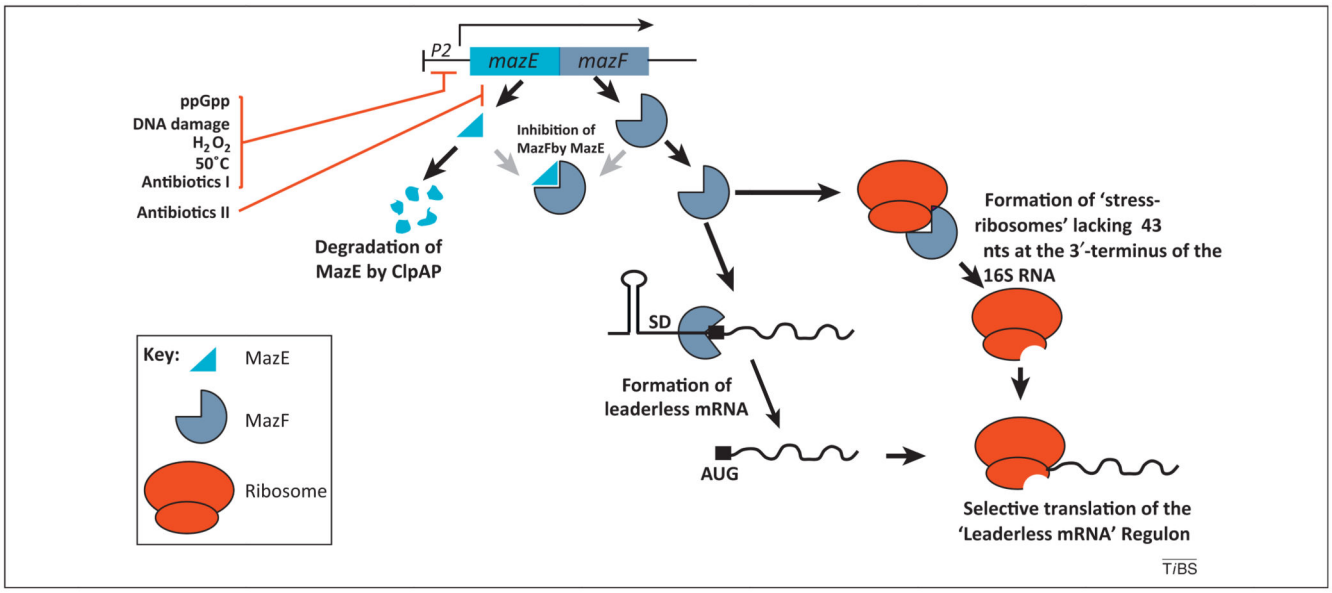


**Figure 1.**

Schematic depiction of the molecular mechanism that leads to selective translation during stress in *Escherichia coli*. Conditions that affect the expression of the *mazEF* module result in rapid degradation of the antitoxin MazE by the ClpAP protease [18,39,40]. Thereby the endoribonuclease MazF is released to exert its toxic effect. Besides degradation of the majority of transcripts [45], MazF removes the 5′-untranslated region (UTR) of specific mRNAs, thus rendering them leaderless. Moreover, it specifically removes the 3′-terminal 43 nucleotides of the 16S rRNA comprising the aSD sequence. As this sequence is of substantial importance for the formation of a translation initiation complex on canonical ribosome-binding sites [48,49], the generated specialized translation machinery selectively translate the leaderless mRNA regulon.




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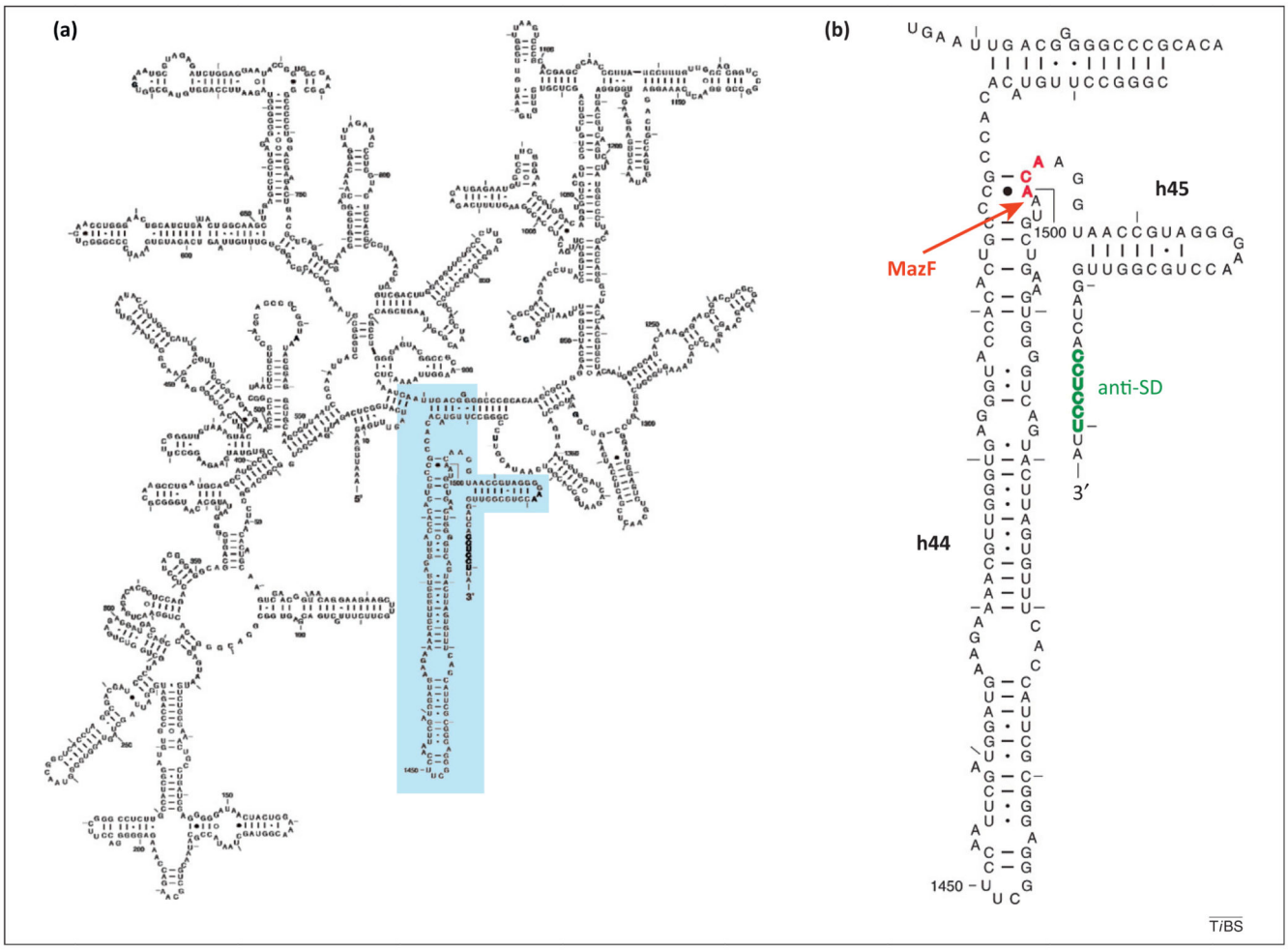


**Figure 2.**

Site of MazF cleavage in the 16S rRNA of assembled and functionally active 70S ribosomes 5′ of nucleotide A1500. **(a)** The secondary structure of the 16S rRNA is shown. The decoding region and helices 44 and 45 that are indicated in light blue are enlarged in **(b)**. The aSD sequence is indicated in green. The MazF recognition motive ACA is shown in red and the site of cleavage is indicated by an arrow.




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