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The stability of an mRNA is influenced by its concentration: a potential physical mechanism to regulate gene expression

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ABSTRACT

Changing mRNA stability is a major post-transcriptional way of controlling gene expression, particularly in newly encountered conditions. As the concentration of mRNA is the result of an equilibrium between transcription and degradation, it is generally assumed that at constant transcription, any change in mRNA concentration is the consequence of mRNA stabilization or destabilization. However, the literature reports many cases of opposite variations in mRNA concentration and stability in bacteria. Here, we analyzed the causal link between the concentration and stability of mRNA in two phylogenetically distant bacteria *Escherichia coli* and *Lactococcus lactis*. Using reporter mRNAs, we showed that modifying the stability of an mRNA had unpredictable effects, either higher or lower, on its concentration, whereas increasing its concentration systematically reduced stability. This inverse relationship between the concentration and stability of mRNA was generalized to native genes at the genome scale in both bacteria. Higher mRNA turnover in the case of higher concentrations appears to be a simple physical mechanism to regulate gene expression in the bacterial kingdom. The consequences for bacterial adaptation of this control of the stability of an mRNA by its concentration are discussed.

INTRODUCTION

Bacteria have to continuously adapt to their changing environment by reprogramming expression of their genes. Understanding how cells regulate gene expression is of major importance to decrypt adaptive phenomena and to understand how cells function in living conditions. Adaptive pathways that contribute to adaptation to newly encountered living conditions are not only controlled at the transcriptional level but also by the level of mRNA stability. For instance, in *Escherichia coli* exposed to osmotic stress, the mRNA levels of *betT*, *proU*, *bmp* were controlled by modulating their stability through the action of RNase III (1–3), and in *Staphylococcus aureus, Mycobacterium tuberculosis* or *Lactococcus lactis*, the induction of stress or starvation caused dramatic stabilization of mRNAs that were at least partly involved in coping with environmental changes (4–6). Control of the stability of mRNA is an adaptive phenomenon and must be considered when analyzing gene expression regulation.

mRNA degradation is an energetically advantageous mechanism for cells to recycle ribonucleotides and to rapidly destroy transcripts that are no longer required for translation. mRNA degradation results from the combined action of endo- and exo-ribonucleases and can be co-transcriptional (7). In *E. coli*, transcripts are degraded via two pathways. The first one begins with internal cleavage by RNase E or RNase G followed by rapid degradation of RNA fragments by exoribonucleases. The second pathway known as the 5′-end-dependent pathway begins by the removal of two of the three phosphate groups of transcriptional start site by the pyrophosphohydrolase RppH (8,9) followed by the action of RNase E and RNase G (10,11). RNase E is involved in a multiprotein complex, the RNA degradosome, by associating with polynucleotide phosphorylase PNPase, RNA helicase B RhlB and enolase (12). In many other bacteria, RNase E has no sequence homolog and is functionally replaced by other ribonucleases, for instance RNase Y (13). In *Bacillus subtilis*, for example, degradation is initiated by internal cleavage by RNase Y and followed by both 3′- and 5′-exonucleases. RNase J, on the other hand, is a bifunctional enzyme with both endonuclease and 5′-exonuclease activities stimulated by RppH-dependent monophosphorylated 5′-ends (14). The two ribonucleases, RNase Y and RNase J, are essential in *B. subtilis* (15,16). RNase J and Y homologs are widely distributed

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in bacterial genomes (17), notably in *L. Lactis*. These two ribonucleases have been shown to play a role in RNA degradation in human pathogens, such as in the gram-negative *Helicobacter pylori* (18) and the gram-positive *S. aureus* (19). The characterization of degradative enzymes identified so far and their phylogenic distribution within the bacterial kingdom suggest that the RNA degradation process is globally conserved even if differences are observed in the nature of RNases and in their time sequence compared to the paradigm *E. coli*.

In addition to the degradation machinery, RNA binding proteins also regulate mRNA turnover. The best characterized regulatory protein is the Host factor I protein (Hfq), which affects the stability of many mRNAs by facilitating base pairing between small RNAs (sRNAs) and the mRNA targets to modulate ribonuclease accessibility (20–24). Another well-known RNA binding protein involved in the regulation of mRNA stability is the carbon storage regulator CsrA (for a review, see Romeo *et al.* (25)). CsrA is a global regulator with multiple targets that binds to the 5′-untranslated region (UTR) and/or early coding sequence of mRNA and alters mRNA turnover, translation and/or transcript elongation (24). A vast, heterogeneous landscape of regulatory RNAs and their associated proteins is emerging to offer a repertoire of regulatory functions which is not yet fully understood (26–29). Ribosomes, RNA binding ribonucleoprotein complexes, can also impede or accelerate mRNA degradation. During translation initiation, the strength of the ribosome binding site (RBS) has been shown to influence mRNA stability in *B. subtilis*, as mutating the RBS to reduce its complementarity to 16s rRNA destabilizes the mRNA, and inversely (30,31). Such behavior appears to be a protective effect of the ribosome against RNase attacks at early steps in translation. Then, during translation elongation, ribosomes also modulate stability, either stabilizing the mRNA by protecting it from RNase attacks, or destabilizing it when translational pausing occurs (for a review, see Deana *et al.* (32)).

Sequence determinants of mRNA molecules, particularly in the 5′- and 3′UTRs, help control stability. 3′UTR sequences harbor transcriptional terminators that stabilize RNA by preventing its degradation by exoribonucleases (33,34). In addition to ribosome binding in the 5′UTR, many sRNAs preferentially interact in this region with the help of Hfq (35) and 5′ terminal stem-loop structures can stabilize transcripts by inhibiting RppH activity (9,11,14,36).

As the concentration of mRNA results from an equilibrium between transcription and degradation, it is generally assumed that if the level of transcription of an individual mRNA remains unchanged, the mRNA concentration will be directly affected by any modification of mRNA stability. Stabilizing a transcript should increase the concentration of mRNA, and conversely, the mRNA concentration should decrease by destabilizing the transcript. Although a large number of mRNAs appear to follow this rule, the reality is more complicated, as examples of modifications of mRNA stability did not result in the expected variation in mRNA concentration (37,38). Bernstein *et al.* analyzed variations in stability at the genome scale in *E. coli* when mutating degradosome components (37). In these mutants, it was assumed that only varying stability would lead to a variation in mRNA abundance, with no major effect of transcription. The majority of genes confirmed the general rule that stabilization and destabilization lead respectively to an increase and a decrease in levels, respectively, but opposite variations in stability and concentration have been observed for several hundreds of mRNAs (37). An extreme opposite relationship was observed for 48 genes with more than a two-fold increase in mRNA half-life with a more than two-fold reduction in concentration. The control of mRNA stability and the consequences for the level of mRNA is thus much more complicated than commonly believed. Generally, when transcripts do not follow the rule, it is assumed to result from a direct or indirect effect on the transcription. But this conclusion has not yet been formally proven. This raises the questions as to whether stability and concentration are intimately associated parameters in mRNA control, and if yes, whether causality is present in this relationship.

To answer this question, we analyzed the relationship between transcript concentration and stability in bacteria using reporter mRNAs. Using selected 5′UTRs from stable mRNAs, we demonstrated that mRNA stabilization either increased or decreased transcript levels, thereby confirming that the level and stability of mRNA are intertwined. We demonstrated that modification of mRNA concentration by only modulating transcription strongly impacted mRNA stability. This showed that the stability of an mRNA mainly depended on its level. A negative relationship between mRNA level and stability was established both for the gram-negative *E. coli* and the gram-positive *L. lactis* bacteria using reporter systems but also validated for native genes at the genome scale. This study demonstrates that the concentration of mRNA is a key parameter in regulating mRNA stability in bacteria.

MATERIALS AND METHODS

Bacterial growth and induction conditions

The strains used in this study are listed in Table 1. Lactococcal strains were routinely grown in M17 medium (39) supplemented with 10 g.l^{-1} glucose (GM17 medium) at 30°C without shaking. *Escherichia coli* strains were grown in Luria-Bertani (LB) broth for cloning steps, at 37°C under shaking. For RNA analysis, cultures were performed in M9 minimal medium supplemented with glucose at 37°C under shaking (40). Antibiotics were used for plasmid maintenance at the following concentrations: erythromycin (5 μg.ml^{-1} for *L. lactis* and 150 μg/ml for *E. coli*), chloramphenicol (5 μg/ml for *L. lactis* and 10 μg/ml for *E. coli*), kanamycin 50 μg.ml^{-1} and ampicillin 100 μg.ml^{-1}for *E. coli*.

Transcription of *lacLM* mRNA in *L. lactis* under the control of P_{nisA} promoter was induced as follows: an overnight culture of MET086 was used to inoculate fresh GM17 medium at initial OD_{580} = 0.1. At OD_{580} = 0.6, a range (0.01, 0.03, 0.1, 0.3, 1 and 10 ng.ml^{-1}) of nisin (Sigma) was added to the cultures and incubated for 1 h. In *E. coli*, transcription of *lacZ* mRNA under the P_{BAD} promoter was induced as follows: an overnight culture of MET346 was used to inoculate fresh M9 medium at OD_{580} = 0.1. At OD_{580} = 0.6, different concentrations of arabinose (Sigma)

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Table 1. Strains and plasmids

Plasmid	plasmid characteristics, content	Reference or source
pVE8065	P_{Zn}-*zitR lacLM* T1T2	(77)
pSEC-Nuc	CmR; PnisA:Nuc	(78)
pIL253	Ery^R;	(79)
pBAD	pBAD-LacZ-cm*yc*-his	LifeScience
p-GST-AS	Amylosuccrase from *Neisseria polysaccharea*	(59)
pMET 047	pIL253; CDS LacLM	This work
pMET 031	pIL253: P_{glnP}-5′UTR *glnP* -LacLM	This work
pMET 032	pIL253: P_{pgk}-5′UTR *pgk* -LacLM	This work
pMET 033	pIL253: P_{rheA}-5′UTR *rheA* -LacLM	This work
pMET 034	pIL253: P_{gadC}-5′UTR *gadC* -LacLM	This work
pMET 035	pIL253: P_{ypiA}-5′UTR *ypiA* -LacLM	This work
pMET 062	pIL253: P_{pgk}- 5′UTR $_{glnP}$ - LacLM	This work
pMET 059	pIL253: P_{pgk}- 5′UTR $_{gadC}$ - LacLM	This work
pMET 060	pIL253: P_{pgk}- 5′UTR $_{ypiA}$ - LacLM	This work
pMET 063	pIL253: P_{pgk}- 5′UTR $_{rheA}$ - LacLM	This work
pMET 086	pIL253: P_{nisA}- UTR $_{gadC}$ - LacLM	This work
pMET 102	P_{BAD}- UTR_{pspA} - AS_{Np}	This work
pMET 104	P_{BAD}- UTR $_{lexA}$ - AS_{Np}	This work
pMET 105	P_{BAD}- UTR $_{patA}$ - AS_{Np}	This work
pMET 107	P_{BAD}- UTR $_{osmC}$ - AS_{Np}	This work
pMET 109	P_{BAD}- UTR $_{yliJ}$ - AS_{Np}	This work
pMET 110	P_{BAD}- UTR $_{tatE}$ - AS_{Np}	This work
Strains	*characteristics*	*Reference or source*
IL1403	L. lactis, plasmid free	(80)
NZ9000	L. lactis MG1363 (nisRK genes into chromosome); plasmid free	(57)
DCT2202	E. coli MG1655 Δ*araFGH*, Ω*pcp18*::*araE533*	(58)
MET345	E. coli DCT2202, ΔlacZ	This work
MET346	E. coli DCT2202, ΔlacZ; contains pBAD-lacZ-*myc*-his	This work
MET086	L. lactis IL1403 contains pMET 086	This work

were added and the culture induced for 30 min. Two runs of cultures were conducted with the same induction level (0.0001%) to interpose the results expressed as fold difference compared to this common condition.

For determination of the mRNA decay in *L. lactis* and *E. coli* transcription was arrested by addition of rifampicin at a final concentration of 500 μg.ml^{-1}. A volume corresponding to 6 mg of cells (dry weight) of culture was withdrawn over time and flash frozen in liquid nitrogen. Six different time points, including the reference sample (before the addition of rifampicin) were used to analyze mRNA degradation kinetics.

Determination of transcriptional start sites

From *L. lactis* IL0403 exponentially growing culture at OD_{580} = 1, total RNAs were extracted as described below and treated as previously described (41). Briefly, 110 μg of total RNA were treated with Turbo DNAse (Ambion-Applied Biosystem) and the absence of contaminating DNA checked by polymerase chain reaction (PCR) using 266-FR.DNA5 primer and a specific primer for each gene whose the transcriptional start site had to be identified (see oligonucleotide Supplementary Table S2). In the first step, RNA adaptor 263-FW.5bt was ligated to 5′monophosphate groups carried by 5′-processed transcripts ends using T4 RNA ligase (New England Biolabs). After removal of excess unligated RNA adaptor by exclusion chromatography using Micro Bio-Spin 30 columns (BioRad), total RNAs were divided in half. One half was subjected to Tobacco Acid Pyrophosphatase (TAP, Epicentre Biotechnologies) and the other half treated without TAP as negative control. The second RNA adaptor 264-FR.RNA5 was added to both in the presence of T4 RNA ligase. The equivalent of 4 μg of RNA was retrotranscribed using random 9-mer primer and the SuperScript III Reverse Transcriptase (Life Technologies) following the supplier's recommendations. Serial dilution of cDNA (1/5 to 1/5000) was then used for identification by PCR using the primer 266-FR.DNA5 combined with a specific oligonucleotide for each gene whose transcriptional start site had to be identified. Amplicons specifically present in cDNA from the library treated with TAP were gel-purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced. The transcriptional start site was determined as the first nucleotide of the targeted transcript ligated to the 3′ end of the 266-FR.DNA5 primer.

Construction of vectors

General DNA manipulation procedures were performed as previously described (42), plasmid DNA was isolated using the QIAprep Miniprep kit (Qiagen). PCR were performed using *Phusion* polymerase (New England Biolabs) and unless otherwise mentioned, were cloned in PCR-Blunt II-Topo vector (LifeScience). Restriction enzymes were purchased from New England Biolabs and used according to the manufacturer's instructions. *lacLM* genes encoding β-galactosidase from *Leuconostoc mesenteroides* subsp. *cremoris* (43) were amplified from pVE8065 (77) without the ATG translational start site and with the addition of BamHI and PstI restriction sites. Regions of selected genes containing the cognate promoter, the 5′UTR and the ATG, were amplified from *L. lactis* IL1403 genomic DNA (BglII/BamHI). All PCR fragments were es-

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tablished in PCR-Blunt II Topo vector (Lifescience) and sequenced. The promoter-5′UTR regions were fused to *lacLM* by ligation at the BamHI site, restoring *lacLM* coding sequence. The expression cassettes were then digested (BglII/PstI), ligated into BglII/PstI digested pIL253 and electro-transformed into *L. lactis* IL1403 competent cells (44).

The different 5′UTR-lacLM cassettes under the control of the P_{pgk} promoter were constructed by PCR assembly. From one side, the P_{pgk} promoter region was amplified by primer 72–5′Pgk-II-BglII and by a set of bipartite oligonucleotides composed of 22 nt corresponding to the sequence upstream from the transcriptional start site of *pgk*, and a sequence from the each 5′UTR starting at the transcriptional start site. From the other side, the 5′UTR-lacLM cassettes were amplified with the reverse complement primer. The two PCR products were gel purified, quantified and mixed. Ten PCR cycles were performed without primers, followed by 25 cycles after addition of external primers (72–5′Pgk-II-BglII and 44–3′LacLM-PstI). Products were cloned, sequenced, extracted by BglII/PstI digestion, ligated in a BglII/PstI digested pIL253 and electro-transferred into *L. lactis* IL1403.

To clone the $5'UTR_{gadC}$-lacLM under the control of nisin promoter, the $5'UTR_{gadC}$-lacLM was PCR amplified (338-UTR-GadC+1 and 44–3′LacLM-PstI). The nisin promoter was amplified using pSEC-Nuc as matrix (232–5′Pnis(BglII) and 335-RW-3′PnisA-1). Equal amounts of gel-purified PCR products were mixed and phosphorylated with 15 U of T4 polynucleotide kinase (New England Biolabs). Phosphorylation of 5′ ends was performed for 30 min at 37°C followed by ligation with 30 U of T4 DNA ligase. The ligation mix was used as matrix for PCR amplification. The PnisA-5′UTRgadC-lacLM cassette was sequenced, gel purified after BglII/PstI digestion and ligated into BamHI/PstI digested pIL253. The resulting plasmid pMET 086 was electro-transferred into the NZ9000 *L. lactis* strain.

The amylosucrase coding sequence from *Neisseria polysaccharea* (AS_{Np}) was fused to the 5′UTR from *yliJ*, *patA*, *lexA*, *tatE*, *osmC* and *pspA* and placed under the expression of P_{BAD} promoter. The AS was PCR amplified with oligonucleotides containing part of the selected 5′UTR on plasmid pGST-AS as a matrix (Table 1). The P_{BAD} promoter was amplified with the remaining part of the 5′UTR (Supplementary Table S2), the cassette P_{BAD}-UTR-ASNp was generated by PCR assembly and cloned in pSEC-Nuc (BglII-XhoI). Resulting plasmids are listed in Table 1.

Deletion of genomic copy of lacZ in *E. coli*

The genomic copy of *lacZ* was deleted using the previously described one-step inactivation method (45). The chloramphenicol resistance gene was amplified on pDK3 with primers extended from the 80 nt homologous to the immediate upstream and downstream coding sequence of *lacZ*. The PCR product was introduced into *E. coli* DLT2202 leading to replacement of *lacZ* by *FRT-Cm-FRT*. The Cm resistance cassette was removed by transitory expression of Flp recombinase from pKD20, leading to strain MET345. The pBAD-*lacZ-cmyc-his* vector (Invitrogen) was introduced to give produce strain MET346.

RNA extraction, quality control and cDNA synthesis

Total RNA was extracted from 6 mg of cells (dry weight). The corresponding volume of culture was frozen in liquid nitrogen and stored at −80°C until extraction. Before cell lysis, each sample was centrifuged for 5 min, at 9900 *g*, at 4°C, washed in 1 ml of TE buffer (Tris-HCl 10 mM, pH8, ethylenediaminetetraacetic acid (EDTA) 1 mM), resuspended in 400 μl of resuspension solution (Glucose 10%, Tris 12.5 mM pH 7.6, EDTA 10 mM). A total of 60 μl of 0.5 M EDTA were added and the mixture was transferred into a tube containing 500 μl of acidic phenol (pH 4.7) and 0.6 g of glass beads. Cells were disrupted at 4°C by 1 cycle of 40 s with a FastPrep-24 instrument (MP Biomedicals). After centrifugation for 5 min, at 16 100 *g*, at 4°C, the aqueous phase was added to 1 ml of TRIzol (Gibco) and incubated for 5 min at room temperature. After addition of 100 μl of chloroform and centrifugation for 5 min, at 16 100 *g*, at 4°C, the aqueous phase was recovered, 200 μl of chloroform were added and the sample centrifuged. The aqueous phase was recovered, 500 μl of isopropanol added and incubated at room temperature 15 min for RNA precipitation. After centrifugation, the pellet was washed with extemporarily prepared 75% ethanol (v/v), dried and resuspended in nuclease-free water. Additional DNase treatment was applied to remove any residual genomic DNA contamination. Total RNA (50 μg) was treated with 7 U of DNase I for 15 min at 20°C followed by a RNA cleanup protocol using RNeasy Mini Kit (Qiagen). The absence of significant genomic DNA contamination was checked by quantitative PCR (qPCR). RNAs were quantified using ND-1000 UV-visible light spectrophotometer (NanoDrop Technologies) and their integrity certified with Bioanalyzer 2100 with the RNA 6000 Nano LabChip kit (Agilent). RNAs were stored at −80°C until required.

Synthesis of cDNA was performed on 10 μg of total RNAs using SuperScript II reverse transcriptase (Life Technologies) as previously described (5). The cDNAs were 1:10 serially diluted and the qPCR was performed in a CFX96 Real Time PCR Detection System (Bio-Rad), using 96-well plate (Bio-Rad) sealed with Microseal 'B' seals (Bio-Rad) as previously described (46). Diluted cDNA from cultures in steady state (without transcription arrest with rifampicin) were used to identify the dilution that would lead to a cycle threshold (Ct) of between 15 and 25. Appropriate dilution was then used for all samples tested (steady state and degradation kinetics).

Primer design and validation

Primers for qPCR were designed using Vector NTI advance v11 (Life Technologies) with a melting temperature of 59–61°C, a length of 20–22 bp and 50–67% GC content. Amplicon sizes ranged from 75 to 148 bp. The reaction efficiency of each pair of primers was tested as a single amplicon on serial dilutions of *L. lactis* or *E. coli* genomic DNA, pMET 032 or pBAD-LacZ-*myc*-his as a matrix, depending on the primer pairs analyzed. The efficiency of validated

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primer pairs was tested on cDNA serial dilutions and focused around 100%. For *L. lactis*, four primer pairs were designed and distributed equally along *lacL*, three along *lacM* (beginning, middle and end of the sequence). For *E. coli*, three primer pairs were similarly designed on *lacZ* and eight on AS_{Np}. For *L. lactis* and *E. coli*, housekeeping genes *tuf* (elongation factor Tu, (47)) and *ihfB* (integration host factor β-subunit, (48)) were used as internal normalization controls, respectively.

High-throughput real-time quantitative PCR

High-throughput real-time qPCR was carried out using the 48.48 or 96.96 dynamic array™ IFCs and the BioMark™ HD System (Fluidigm Corporation, CA, USA) following the manufacturer's protocol (49). Briefly, the steps were as follows. Fourteen pre-amplification cycles were performed with a pooled primer mixture (0.2 μM). The pre-amplified samples were treated with 8 U of exonuclease I (New England BioLabs), diluted 1:5 with Tris-EDTA buffer and added to a 'Sample Mix' consisting of TaqMan® Gene expression Master Mix (Applied Biosystems), DNA Binding Dye Sample Loading Reagent (Fluidigm), EvaGreen® dye (Biotium) plus Tris-EDTA buffer, as recommended. In parallel, each primer pair (20 μM) was added to a 'Primer Mix' composed of Assay Loading Reagent (Fluidigm) plus Tris-EDTA buffer, as recommended. An Integrated Fluidics Circuits (IFC) controller was used to prime the fluidics array, then 5 μl of each sample and primer mix were loaded in the appropriate inlets. The loaded chip was transferred to the BioMark™ HD System and qPCR was performed using the following temperature program: 2 min at 50°C, 30 min at 70°C and 10 min at 25°C; followed by a hot start 2 min at 50°C, 2 min; then 10 min activation at 95°C for 35 PCR cycles of 15 s at 95°C for denaturation, and 60 s at 60°C for annealing and elongation. The melting curve analysis consisted of 3 s at 60°C followed by heating to 95°C with a ramp rate of 1°C/3 s. To determine the mRNA concentration in steady state, each sample was loaded from 3 to 5 times in the array and each primer pair was loaded from 3 to 18 times, leading to 9 to 90 quantitative technical replicates for each biological sample. mRNA decay was similarly quantified leading to 3 to 18 technical replicates for each biological sample in the degradation kinetics and for each primer pair.

Data analysis and statistical treatment

For quantifications by qPCR in plates, the Ct values were determined with a baseline at 100 relative fluorescent units above the background. For quantification by BioMark system, the automatically determined baseline was selected. For direct comparison of strains or of culture conditions for the same strain, results were expressed as differences (n-fold) between either strains or culture conditions, referred to the control strain (with UTR from *pgk*) or to an inducer concentration (nisin or arabinose). The Pfaffl analysis method was applied (50), considering the ΔCt ratio between strains (or conditions), exclusively for the same primer pair. Results are expressed as means of n-fold with standard deviation of the mean. To determine the half-life of mRNA, for each condition analyzed and by one technical replicate of each primer pair, Ct were plotted as a function of the time after addition of rifampicin. Since the Ct values were very sensitive to small changes in concentration, we estimated that it was not possible to accurately estimate any delay in transcript degradation after rifampicin addition (7). Therefore the mRNA half-lives ($t_{1/2}$) were calculated from the degradation rate constant (k) corresponding to the slope of the plot of the Ct as a function of time with the relation $t_{1/2} = 1/k$. Only slopes with a $R^2 > 0.75$ were considered. The $t_{1/2}$ measured for each repetition and each primer pair are expressed as the mean, and the standard deviation of the mean was calculated. Data at the genome scale (transcriptome and stabilome) were previously published (40,51) and reanalyzed to focus on the stability and concentration relationship for this study.

RESULTS

Stabilization of an mRNA had an unpredictable effect on its concentration

The stabilome (omics measurement of all mRNA half-lives) of *L. lactis* was previously determined in exponentially growing cells and showed that the transcript half-lives were widely distributed (5). About 90% of half-lives ranged from 1.4 to 10 min (median half-life of 5.4 min) but some transcripts were very stable with half-lives up to 15 min. To analyze the effect of stabilizing a particular mRNA on its intracellular level, we selected four mRNA with half-lives higher than the median value in the stabilome, present as a single transcript unit or being at the first position in operonic structures and with a concentration corresponding to the average of the whole transcriptome (5). The selected genes *glnP*, *gadC*, *ypiA* and *rheA* displayed a half-life of 6.3, 18.9, 21 and 31 min, respectively (Figure 1A). In addition, *pgk* was selected as reference gene, with an mRNA half-life of 5.5 min; i.e. close to the stabilome median value. In *L. lactis*, the UTRs have not been formally identified and only a few transcriptional start sites have been determined experimentally (52–54). We experimentally determined the transcriptional start sites of the selected genes using the Tag-RACE method (41), defining the 5′UTRs to be 73, 149, 66, 26 and 102 nt long for *pgk*, *glnP*, *gadC*, *ypiA* and *rheA*, respectively (Supplementary Table S1). Each 5′UTR was cloned between the transcriptional control of the P_{pgk} promoter and the *lacLM* reporter gene, encoding a β-galactosidase from *Leunonostoc* sp. active in *L. lactis* (43) leading to the constructions P_{pgk}-$5'UTR_{\text{selected gene}}$-*lacLM*. Plasmids were introduced in *L. lactis* IL1403 and the $5'UTR_{\text{selected gene}}$-*lacLM* mRNA half-lives were measured by qRT-PCR in exponentially growing cells. As shown in Figure 1A, the $t_{1/2}$ of the $5'UTR_{pgk}$-*lacLM* mRNA was 3 min, compared to 5.5 min for the native chromosomally encoded *pgk* mRNA. The difference in the $t_{1/2}$ values could be due to either stability information carried by the coding sequence moiety and the 3′UTR of the mRNA and not only the 5′UTR, and/or by the difference in mRNA concentration between native *pgk* mRNA and $5'UTR_{pgk}$-*lacLM* mRNA because of the plasmid carriage of the latter. For the four other $5'UTR_{\text{selected gene}}$ tested, we ob-

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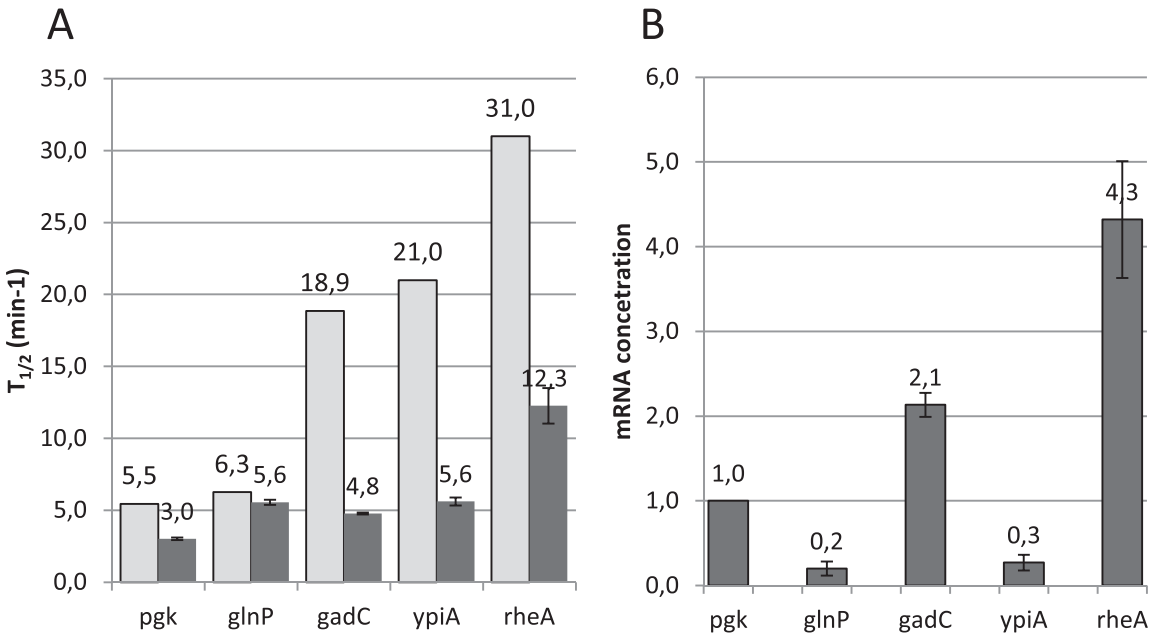

Figure 1. Influence of the 5′UTR on half-lives in *Lactococcus lactis*. (**A**) The mRNA half-lives of native selected gene (*pgk*, *glnP*, *gadC*, *ypiA* and *rheA*) from stabilome data (5) (light gray) and of reporter mRNA composed of *lacLM* flanked with the 5′UTR of these selected genes and under the transcriptional control of the P_{pgk} promoter (dark gray). (**B**) Concentration of *lacLM* mRNA under the P_{pgk} promoter when flanked with the 5′UTR from genes mentioned on the abscisses, expressed as fold change compared to the reference *5′UTR$_{pgk}$-LacLM* mRNA. Error bars represent standard deviation of the mean.

served stabilization of *5′UTR*$_{\text{selected gene}}$*-lacLM* mRNA with a $t_{1/2}$ varying from 4.8 to 12.3 min, compared to the reference *5′UTR$_{pgk}$-lacLM* at 3 min. The $t_{1/2}$ measured here or by stabilome showed that there was no correlation between the length of the 5′UTR and the mRNA stability either for the native gene or the *lacLM* reporter. The smallest 5′UTR (*ypiA*, 26 nt) and the longest 5′UTR (*glnP*, 149 nt) showed similar stability, excluding the length of the 5′UTR as the key determinant of stability in these mRNAs.

For the five P_{pgk}-*5′UTR*$_{\text{selected gene}}$*-lacLM* constructs, the mRNA concentration was expected to be similar since the same promoter was used. Therefore any variation in mRNA concentration would be due in principle to variation in stability. As expected by stabilizing the transcript, the concentrations were increased in *5′UTR$_{gadC}$-lacLM* and *5′UTR$_{rheA}$-lacLM* mRNAs (Figure 1B). However the opposite was observed in *5′UTR$_{glnP}$-lacLM* and *5′UTR$_{ypiA}$-lacLM* mRNAs (Figure 1B). This could be linked to the influence of the DNA sequence of the 5′UTR used on the transcription level of P_{pgk}, as similarly documented with synthetic or point mutations near the transcriptional start site, which enhanced or reduced the promoter activity (55,56). These results showed that modulating the stability of mRNA by the use of different 5′UTR sequences modified not only the mRNA level, but probably the expression level. This made it very difficult to isolate just the effect of stability on the mRNA level and is therefore more appropriate for studying the relationship between mRNA level and stability to modulate the expression level.

Increasing the mRNA level by transcription modulation reduces its stability

To analyze the stability of a reporter mRNA at different transcription levels, the stability of each *5′UTR*$_{\text{selected gene}}$*-lacLM* mRNA was analyzed when expressed under the control of its cognate promoter. The promoter region and 5′UTR of the selected genes were fused to the *lacLM* gene and plasmids were introduced in *L. lactis* IL1403. The *5′UTR*$_{\text{selected gene}}$*-lacLM* mRNA remained stable with increased $t_{1/2}$ compared to the *5′UTR$_{pgk}$-lacLM* reference for all constructs (Figure 2A). The $t_{1/2}$ value of the mRNA was lower than the $t_{1/2}$ of their chromosomally encoded counterpart, when transcribed from P_{pgk} or from its cognate promoter for three constructions *5′UTR$_{gadC}$-lacLM*, *5′UTR$_{ypiA}$-lacLM* and *5′UTR$_{rheA}$-lacLM*. This suggests that the transcription level also influences stability. The concentrations of *5′UTR*$_{\text{selected gene}}$*-lacLM* mRNA measured in each strain differed significantly (Figure 2B). All the native promoters were weaker than the P_{pgk} promoter (Figures 1B and 2B). We plotted the mRNA half-life as a function of its concentration when expressed either under the control of P_{pgk} or the cognate promoter (Figure 3). For *5′UTR$_{gadC}$-lacLM*, *5′UTR$_{ypiA}$-lacLM* and *5′UTR$_{rheA}$-lacLM*, when the promoter exchanges led to an increase in mRNA concentration, a concomitant decrease in $t_{1/2}$ was observed. For *5′UTR$_{glnP}$-lacLM*, the two promoters led to the same mRNA concentration and the same $t_{1/2}$ (overlapping dots in the Figure 3). These results reinforced the hypothesis that mRNA level and stability were linked parameters, independently of the mRNA sequence and suggests that an increase in a transcript concentration favors its destabilization.

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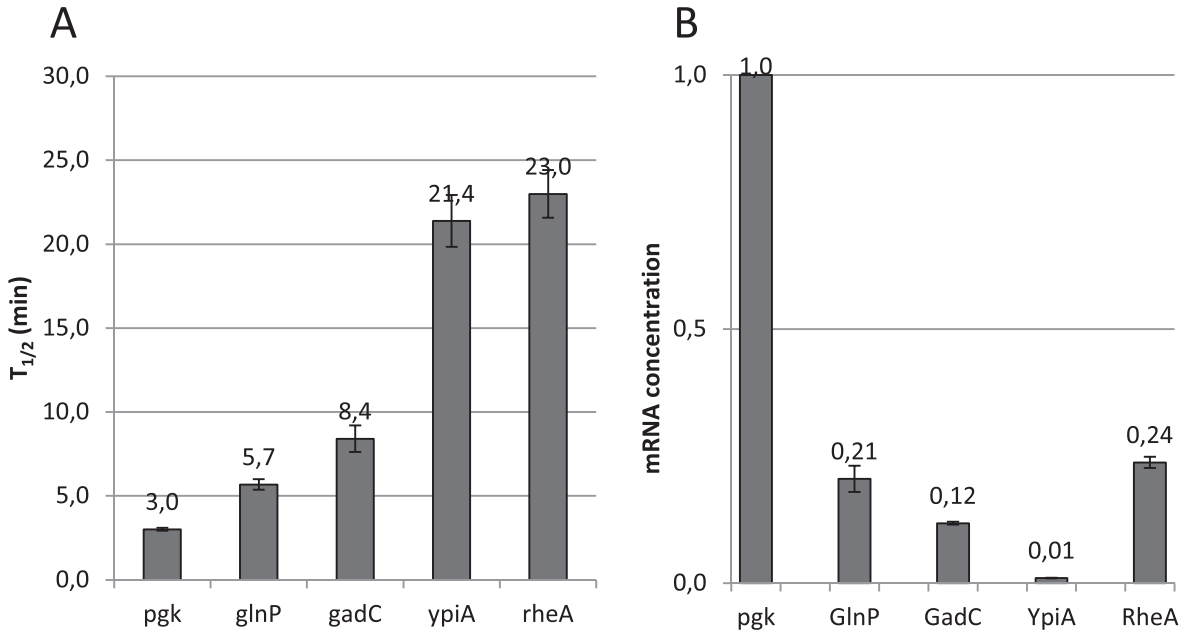

Figure 2. Influence of the transcription level on half-lives in *Lactococcus lactis*. The lacLM coding sequence was fused to the different 5′UTR and their native promoter mentioned on the abscisses and expressed in *L. lactis*. The mRNA half-lives (**A**) and concentration (**B**) were measured. The concentration of *lacLM* mRNA is expressed as fold change compared to that of $5'UTR_{pgk}$-LacLM. Error bars represent standard deviation of the mean.

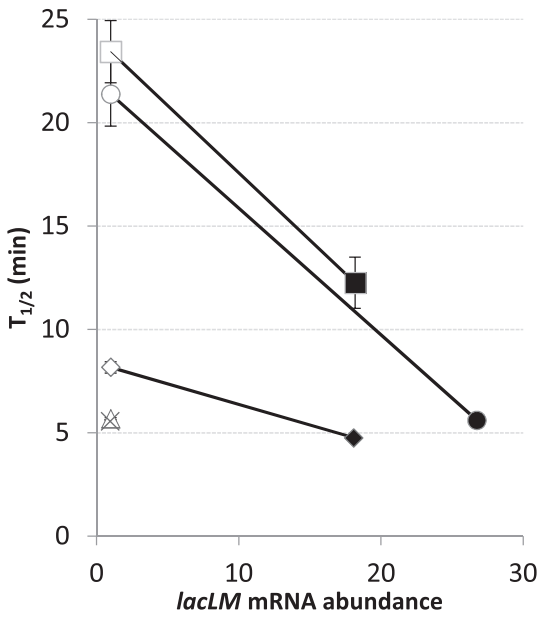

Figure 3. Variation in *lacLM* mRNA half-lives as a function of its concentration in *Lactococcus lactis*. The 5′UTR of *gadC* (diamonds), *rheA* (squares), *ypiA* (circles) and *glnP* (triangles) were selected. Two promoters for each selected UTR were used to modulate the mRNA concentration: the P_{pgk} promoter (filled symbol) and the native promoter (empty symbol). The concentration of each 5′UTR-lacLM mRNA expressed under the control of P_{pgk} is expressed as fold difference compared to its expression under the control of its native promoter. When transcribed from P_{pgk} or P_{glnP}, the *5′UTR$_{glnP}$-lacLM* mRNA displayed similar concentrations and $t_{1/2}$ values, leading to superimposed points on the graph (open triangle for P_{pgk}, cross for P_{glnP}). Error bars represent standard deviation of the mean.

To confirm the negative relationship between mRNA concentration and stability, a wider range of mRNA concentrations was scanned for the *5′UTR$_{gadC}$-lacLM* transcript using the nisin inducible promoter (57). The P_{nisA}-$5'UTR_{gadC}$-*lacLM* expression cassette was introduced in *L. lactis* NZ9000 strain carrying chromosomally encoded *nisRK* genes required for linear induction of the P_{nisA} promoter by nisin. Exponentially growing cells were induced for one hour at six different nisin concentrations ranging from 0.01 ng.ml^{-1} to 10 ng.ml^{-1}. The highest induction level resulted in a 100-fold increase in *lacLM* mRNA abundance compared to the lowest. The half-life of *5′UTR$_{gadC}$-lacLM* mRNA was determined at each induction level and plotted as a function of the mRNA concentration (Figure 4). The negative relationship between mRNA level and stability observed using different promoters was confirmed. At the lowest induction level, the $t_{1/2}$ was about 18 min and dropped to about 3 min at the highest induction level. As a tendency, at low mRNA concentrations, a slight increase in concentration resulted in a sharp decrease in stability whereas at high concentrations, variations in the concentration of mRNA had less impact on stability. The nisin system was at its maximum induction level at 1 ng.ml^{-1} as the concentration was similar to that measured at 10 ng.ml^{-1} of nisin. At these two induction levels, the associated $t_{1/2}$ were also similar (3.2 and 3.6 min for 1 ng.ml^{-1} and 10 ng.ml^{-1}, respectively), reinforcing the hypothesis of a direct effect of mRNA concentration on the stability of the mRNA. The measure of $t_{1/2}$ and concentration of *LacLM* mRNA was obtained with seven different primer pairs distributed along the transcript which gave same results (data not shown), confirming that the increased stability at low mRNA concentration was not an artifact due to the accumulation of degradation products.

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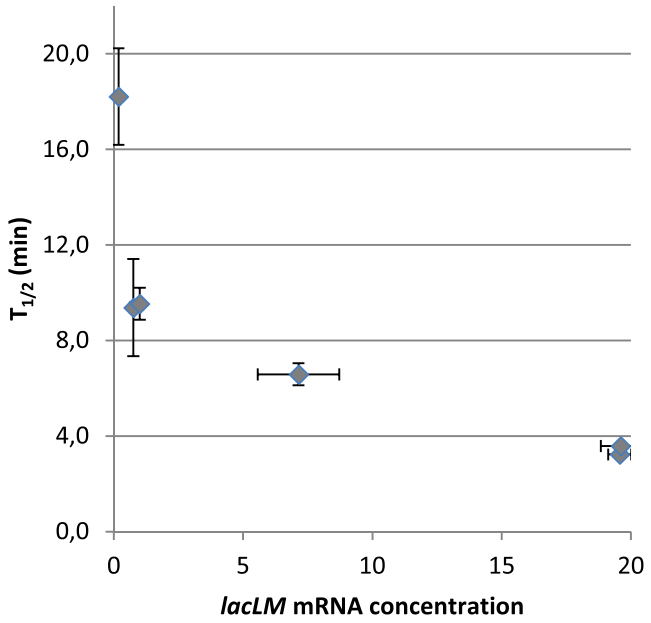

Figure 4. Large scale variation in the concentration on *lacLM* mRNA half-life in *Lactococcus lactis*. The $5'UTR_{gadC}$-*LacLM* mRNA was expressed under the control of the P_{nisA} inducible promoter. Different amounts of nisin (0.01 to 10 to ng.ml^{-1}), were used to scan a wide range of expression. The mRNA concentration and $t_{1/2}$ were measured. mRNA concentration is expressed as fold change compared to the reference culture induced at 0.1 ng.ml^{-1}. Error bars represent standard deviation of the mean ($15 < n < 56$ for $t_{1/2}$; $n = 7$ for concentration).

Negative mRNA level—stability relationship exists in different bacteria

Is the negative relationship between transcript level and stability restricted to *L. lactis* or is it a general rule in the bacterial kingdom? To answer this question, we analyzed the link between mRNA level and stability in *E. coli* MG1655-based strain DLT2204 (58). In this strain, we deleted the chromosomal copy of *lacZ* and introduced a *lacZ* plasmidic copy under the transcriptional control of the arabinose inducible P_{BAD} promoter. Exponentially growing cultures were induced with seven different arabinose concentrations, (0.00001–0.0003%) to cover a 30-fold inductor range. Total RNAs were extracted and *lacZ* mRNA was quantified by qRT-PCR. The highest induction level resulted in a 27-fold increase in *lacZ* mRNA abundance compared to the lowest. For each induction level, the *lacZ* mRNA $t_{1/2}$ was determined and ranged from 6.3 to 1.2 min. For all conditions, mRNA concentration and stability were determined with three different primer pairs which gave same results, confirming that the variations observed correspond to functional mRNA and not to mRNA decay products accumulation. Plotting the mRNA half-life as a function of the concentration revealed a similar negative relationship for *E. coli* as the one observed for *L. lactis* (Figure 5A). Increasing only the concentration of a specific mRNA also reduced its stability in *E. coli*. Again, at the lowest concentrations, a moderate increase in mRNA concentration resulted in a sharp decrease in half-life whereas at high concentrations, the decrease of the half-life was reduced and appeared to reach an asymptote. We concluded that the negative mRNA level-stability relationship was indeed a shared phenomenon in *L. lactis* and *E. coli*, two distantly related bacteria, and could exist in other micro-organisms.

In this system, only the transcription level of *lacZ* was modified for a short period. Nevertheless, one can speculate whether the observed decrease in stability is linked to changes in the level of degradation machinery. We analyzed the mRNA level of a large panel of enzymes related to mRNA degradation. For each induction level tested, we quantified the transcript concentrations of endoribonucleases (RNaseE, RNaseG, RNaseI, RNAseIII), exoribonucleases (RNaseR, PNPase, RNaseII, RNaseD, RNAseT, Orn) and enzymes related to RNA degradation (PAP-I, Eno, RhlB, RhlA, Hfq, etc.). The transcript level was not significantly modified by the induction of the reporter *lacZ* mRNA in any of these genes (Supplementary Figure S1). We also checked that the expression of genes coding for proteins related to RNA metabolism but not to RNA degradation (DeaD, RNaseP, CspA) and well known controls (CsdA, IdnT, HcaT) did not change in the experiment (Supplementary Figure S1). We can thus conclude that the reduction of the $t_{1/2}$ of *lacZ* mRNA observed cannot be attributed to changes at the expression level of the RNA degradation machinery.

These results were based on the use of a plasmid inducible expression system. To exclude the possibility that a bias could have been introduced by this system, we analyzed the half-life of the native chromosomally expressed *lacZ* mRNA in the *E. coli* MG1655 reference strain. Values were extracted from previously obtained stabilomic data ((40), Dressaire *et al.*, unpublished data). As shown in Figure 5B, the negative relationship between native *lacZ* mRNA concentration and stability was still observed at the scale of a single copy chromosomally encoded native gene. This result excluded any effect either of the plasmid carriage of the reporter gene or the use of transcriptional inducer on the observed correlation. Taken together, these results showed that the intracellular concentration of an mRNA played a role in its own stability. The more abundant an mRNA, the less stable it was, and this relationship was not restricted to a specific microorganism. We concluded that the mRNA level can be considered as a parameter that actively controls mRNA stability.

We extended the analysis to another reporter gene, the amylosucrase (AS_{Np}) from *Neisseria polysaccharea* (59). We selected 6 different 5′UTR from *E. coli* (with length from 28 to 74 nt) and inserted them between the P_{BAD} promoter and the AS_{Np} coding sequence. The AS_{Np} transcription was induced for each construction by addition of arabinose and the $UTR_{selected}$-AS_{Np} mRNA concentration and stability were determined as described above. As shown in Figure 5C, the relationship between concentration and stability of AS_{Np} was similar to the one of *lacZ*. Increasing the concentration decreased the stability. The similar relationship between concentration and stability found again with another reporter excludes that it is a gene-specific response.

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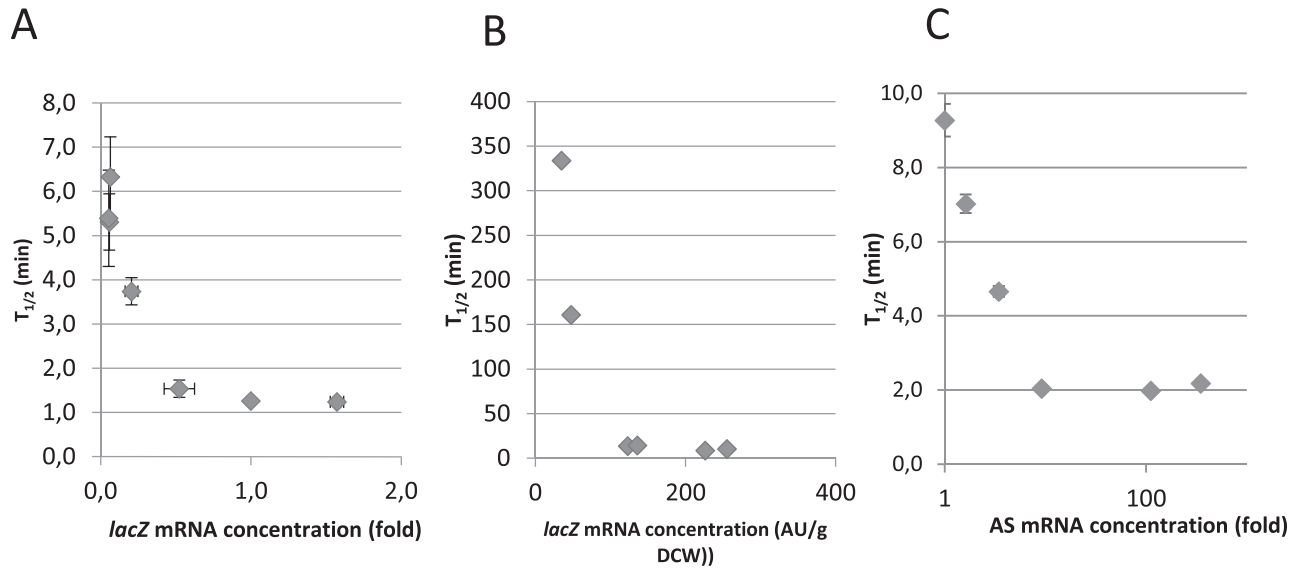

Figure 5. Relationship between *lacZ* mRNA concentration and half-life in *Escherichia coli*. (**A**) The transcription of *lacZ* under the P_{BAD} promoter was induced by addition of different concentrations of arabinose. The *lacZ* mRNA concentration is expressed as fold change compared to the culture induced at 0.0001% arabinose. The half-lives are plotted as a function of mRNA concentration. Error bars represent standard deviation of the mean ($6 < n < 13$ for $t_{1/2}$; $25 < n < 42$ for concentration). (**B**) The half-lives of *lacZ* mRNA plotted as a function of the *in vivo* concentration when the chromosomal native gene was expressed in *E. coli* cells growing in different conditions. *lacZ* mRNA concentrations are expressed in arbitrary units per gram of dry cell weight. (**C**) The transcription of AS_{NP} fused to different 5′UTR and under the P_{BAD} promoter was induced with 0.001% arabinose. The 5′UTR fused to AS_{NP} coding sequence are those from *yliJ*, *patA*, *lexA*, *tatE*, *osmC* and *pspA*, from the left to the right. The AS_{NP} mRNA concentration is expressed as fold change compared to the strain expressing AS_{NP} at the lowest concentration. The half-lives are plotted as a function of mRNA concentration. Half-lives and concentrations were determined with 8 primer pairs distributed along the AS_{Np} coding sequence. Error bars represent standard deviation of the mean ($5 < n < 30$ for $t_{1/2}$; $38 < n < 62$ for concentration).

Negative mRNA level—stability relationship extended to the genome scale

To investigate whether the negative relationship between mRNA level and stability could be extended to other native genes in *in vivo* contexts, we re-computed transcriptomic and stabilomic data previously obtained for *L. lactis* (52) and *E. coli* ((40,51) and unpublished data). In both microorganisms, the transcriptome and the stabilome were measured in different environmental conditions leading to *in vivo* variations in mRNA concentrations and half-lives. We then used these data to analyze the relationship between the mRNA level and half-lives at the omics scale for all the native genes.

First, we observed that the negative relationship between mRNA level and stability was confirmed at the level of functional categories as exemplified by mRNAs of ribosomal proteins (Figure 6). These very abundant proteins are considered to play central role in the life of a cell. For the different mRNAs in this particular functional category, negative relationships, especially at very low mRNA concentrations, can be drawn between the *in vivo* levels of native mRNAs and their stability in *L. lactis* (Figure 6A) and *E. coli* (Figure 6B). We then extended the analysis to the entire populations of mRNAs in the two bacteria models (Figure 7A and B). Considering 1633 and 4068 native mRNAs in *L. lactis* and *E. coli*, respectively, the negative relationship was conserved at the genome scale in both microorganisms. Like in the reporter systems, it was still at the lower mRNA concentrations that the stability sensitivity to concentration changes was the highest. Nevertheless, in the case of native mRNAs at low concentrations, this sensitivity appeared to be more pronounced in *E. coli* than in *L. lactis*, probably due to specificities of the degradation process in the two bacteria.

DISCUSSION

mRNA concentration is a key determinant of stability

In natural or artificial environments, bacteria have to face changing living conditions. To respond to environment modifications, they reprogram their gene expression to adjust to metabolic requirements and/or available resources. In addition to modification of transcription, the regulation of mRNA stability is a key mechanism in regulating gene expression. In steady state, mRNA homeostasis is maintained by keeping the degradation rate the same as the transcription rate. It is widely assumed that at a constant transcription rate, the stabilization of a transcript should increase its concentration and conversely, its destabilization should reduce it. However, the reality seems to be much more complex, as examples have been reported where the destabilization of a transcript was associated with an increase in mRNA level, even if a constant transcription rate was assumed (37,38). To analyze the mRNA concentration-stability relationship, we used 5′UTRs selected from stable genes in *L. lactis*, fused to a *lacLM* reporter gene to measure the effect of changes in stability on mRNA concentration. We first confirmed that the use of 5′UTRs from stable transcripts stabilized a reporter mRNA, thereby confirming that part of the turnover information is determined by

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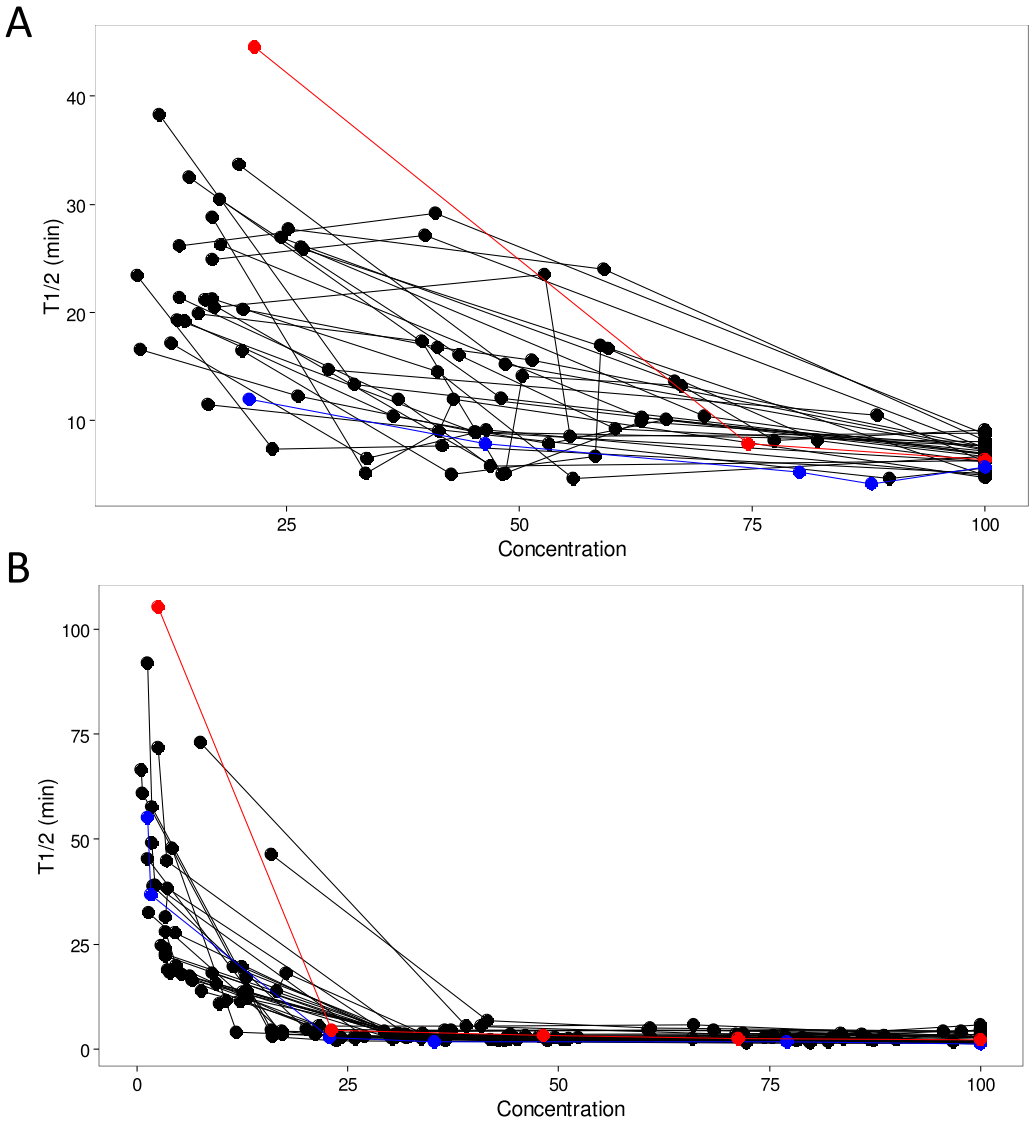

Figure 6. Relationships between mRNA concentration and the stability of ribosomal proteins. Plots represent the mRNA half-lives of a ribosomal protein as a function of the concentrations expressed as a percentage of the highest concentration. The mRNA half-lives and concentrations were determined by stabilome and transcriptome experiments, respectively. (**A**) For *Lactococcus lactis*, 28 plots with at least two experimental stabilome and transcriptome measurements in four environmental conditions (leading to growth rates of 0.09, 0.24, 0.35 and 0.47 h^{-1} (51)) are depicted. List of the 28 genes of ribosomal proteins used for this figure: *rplA, rplI, rplM, rplO, rplQ, rplS, rplT, rpmB, rpmE, rpmF, rpmGB, rpmI, rpmJ, rpsA, rpsB, rpsD, rpsF, rpsG, rpsH, rpsI, rpsK, rpsL, rpsM, rpsO, rpsP, rpsT, rpsU* and *ylxQ*. The *rpsO* and *rpsP* mRNAs displaying the highest and the lowest half-lives are depicted in red and blue, respectively. (**B**) For *Escherichia coli*, 31 plots with at least five experimental stabilome and transcriptome measurements in six environmental conditions (corresponding to growth rates of 0.04, 0.11, 0.38, 0.51 and 0.80 h^{-1} and stationary phase; (40), unpublished data) are presented. List of the 31 genes of ribosomal proteins used for this figure: *rplA, rplE, rplF, rplJ, rplK, rplL, rplM, rplN, rplQ, rplS, rplT, rplU, rplX, rpmA, rpmB, rpmC, rpmE, rpmF, rpmG, rpmH, rpmI, rpsB, rpsG, rpsH, rpsI, rpsL, rpsN, rpsO, rpsP, rpsT* and *rpsU*. The *rpmA* and *rplK* mRNAs displaying the highest and the lowest half-lives are depicted in red and blue, respectively.

the sequence and/or the structure of the 5′UTR, independently of the downstream mRNA sequence (60–62). In addition, differences in the mRNA level were demonstrated between constructs although they were transcribed by the same P_{pgk} promoter. For the four *5′UTR*$_{\text{selected gene}}$*-lacLM* mRNAs analyzed, two followed the widely assumed pattern of mRNA stabilization leading to increased concentration (*5′UTR*$_{gadC}$*-lacLM* and *5′UTR*$_{rheA}$*-lacLM*). But for the two others (*5′UTR*$_{glnP}$*-lacLM* and *5′UTR*$_{ypiA}$*-lacLM*), transcript stabilization led to decreased mRNA level. This result suggests that in this case the decrease in mRNA concentration was likely related to variation in rates of transcription initiation due to the modification of the promoter environment in the different 5′UTR sequences. In conclusion, due to the complexity of the direct and indirect role of 5′UTR on transcription level, it is almost impossible to predict the effect of the modification of mRNA stability on its concentration.

To isolate only the effect of stability on mRNA level, we modulated the concentration of specific mRNAs by using

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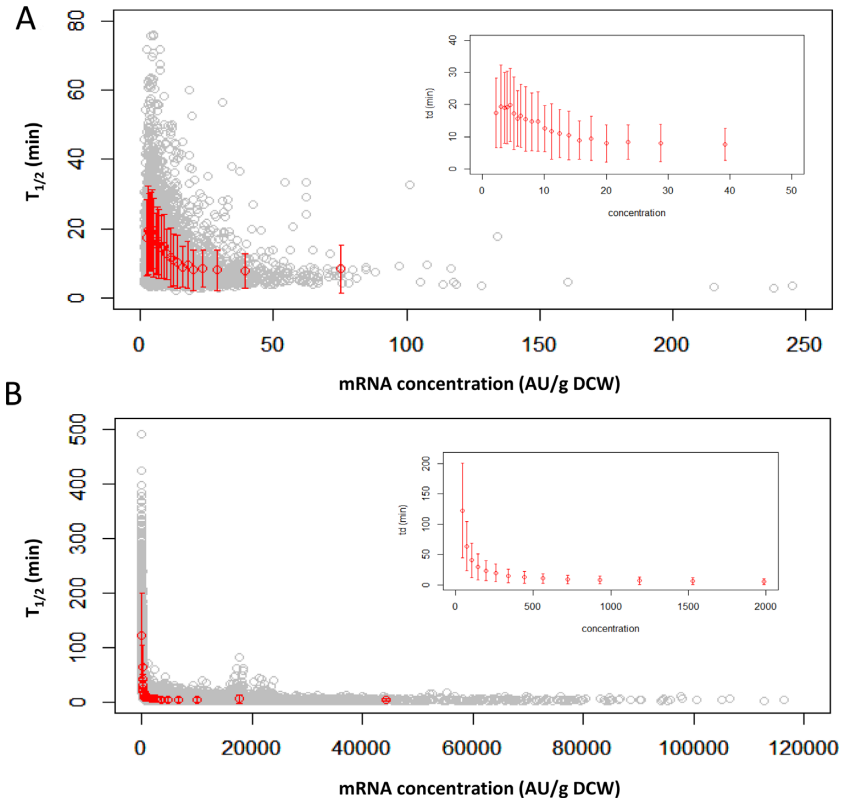

Figure 7. Relationship between mRNA concentration and stability at the genome scale. Plots represent mRNA half-lives as a function of concentration (in arbitrary units per gram of dry cell weight) of the whole population of native mRNAs. The insert is a zoom on the low concentrations. (**A**) For *Lactococcus lactis*, the plot represents stabilome and transcriptome measurements of 1633 genes in four growth conditions (51). In red, the means ± the standard deviation for every 200 values are depicted. (**B**) For *Escherichia coli*, the plot represents stabilome and transcriptome measurements of 4068 genes in six growth conditions ((40) and unpublished data). In red, the means ± the standard deviation for every 1000 values are depicted.

different promoters. There was a negative relationship, as increasing the concentration led to the destabilization of the transcripts. This was shown for four different mRNAs using native promoters and confirmed using inducible promoters and a wide range of mRNA concentrations. The negative relationship was determined in *L. lactis* and *E. coli*, two phylogenetically distant bacteria. The lack of a homolog of RNaseE in *L. lactis* supports the hypothesis that the negative relationship is a general feature in bacteria. We checked that in *E. coli*, the transcription of the degradation machinery remained unchanged. Increasing the mRNA concentration thus accelerated the mRNA turnover without modifying degradative capacities of the cell in terms of degradation machinery levels. The mRNA concentration *per se* can thus be considered as a key determinant of mRNA stability.

Possible role in translation

The negative effect of mRNA concentration on stability is not incompatible with the positive correlation between mRNA level and the amount of protein produced. In both *L. lactis* and *E. coli*, increasing the concentrations of *lacLM* and *lacZ* mRNAs increased the production of the β-galactosidase protein even though the stability of the mRNAs was reduced (not shown). We previously demonstrated in *L. lactis* that the most unstable mRNAs were those loaded with the highest number of ribosomes (63). In other words, an abundant mRNA may be highly translated even if it is unstable. For the physiology of the cell, this can be a way to control the protein synthesis. When the cells need to rapidly accommodate protein levels, it could be deleterious to have highly translated stable mRNAs. In adaptation or stress response, this would result in continued production of proteins that are no longer required. The translation level is an important contributor to the relationship between abundance and stability of mRNA. Ribosomes may act either as a protective barrier against RNases. Modification of ribosome binding, premature translation termination and translation pausing are known to trigger significant mRNA destabilization (30,31,64,65) or as an helper to recruit the mRNA-degrading machinery (18,66). Highly or poorly translated mRNAs might therefore be differently degraded. The relationship between the translational status of an mRNA and its stability awaits experimental verification.

The mechanism of control of the mRNA concentration-stability correlation remains unknown

Another angle of analysis is to simply consider the interaction between mRNA concentration and its degradation as the equivalent of an enzyme–substrate interaction. The situation is much more complicated than a 'classical' enzymatic reaction where one enzyme has to deal with one or a few substrates. For RNA degradation, different en-

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zymes with specificities and overlapping activities have to deal with thousands of different substrates simultaneously. Here we demonstrated that at high concentration, mRNAs were destabilized. *In vitro* measurements of kinetics studies of RNase E and RNase G activities confirmed that the enzymes can be saturated at high substrate concentration with a Km of about 1 μM (67,68). *In vivo*, the total mRNA concentration was estimated to about 1 mM (CyberCell Database), a substrate concentration high enough to saturate the RNases E and G. But when considering individually each mRNA, the concentration may be lower than 1 μM (0.25 μM in average considering the presence of 4000 mRNA). Therefore, an increase in concentration of this mRNA will lead to an increase of its degradation because of a higher probability of RNase binding.

In that case, stability regulation is the result of a simple physical mechanism. In *E. coli*, the RNase E is membrane-associated and the mRNAs have to access the membrane for their decay (69). The dynamics of degradosome in the inner cytoplasmic membrane was recently demonstrated to be strongly affected by a reduction in mRNA concentration (70), thereby corroborating the major influence of mRNA concentration on its stability demonstrated in this work. Delocalization of RNase E from the membrane to the cytoplasm resulted in slow growth that was suspected to result from a defect in accessibility of RNase E to its substrates, either through its role in mRNA decay or indirectly through other processes involving RNase E such as rRNA and tRNA maturation (71). Similar effects were observed with the delocalization of the major membrane associated endoribonuclease RNase Y in *Staphylococcus aureus* (72). Considering our results, one can hypothesize that the negative relationship between mRNA concentration and stability would be affected in a strain with delocalized RNase E, because of modified accessibility of RNase E to its substrates (72,73). Further investigations are required to evaluate this assumption, particularly for mRNAs at very low concentrations.

Physiological role

What is the physiological role of the negative relationship between mRNA concentration and stability? In addition to reporter mRNAs, the relationship was validated at the genome scale for native mRNAs with destabilization of the transcripts with an increase in the concentration of cells adapted to different environments. In these conditions, the mRNA homeostasis must reach an equilibrium imposed by the environment and the energy status of the cell. When resources are available to sustain a high growth rate, the mRNA turnover is rapid. When resources are limited or in the case of extreme adaptive conditions, mRNA stabilization might be a way for the cell to reduce the energetic cost associated with their turnover. When *B. subtilis* encounters nutrition limitation and sporulates, the rRNA and mRNA contents are reduced by modifying the transcription rate and the degradation profile (74). However, the spores have to preserve a minimum amount of rRNAs and mRNAs to resolve dormancy when appropriate environmental conditions are restored. A similar scenario has been observed in *M. tuberculosis* (75). In dormant cells, total mRNA content decreased and a cohort of mRNAs was stabilized, particularly those coding for biosynthetic enzymes, adaptation process and initiation of transcription. Stable mRNAs, even if they are not abundant in the cell, could represent the minimum readily translatable mRNA pool for resuscitation in appropriate environmental conditions. This has been suggested by analyzing the stability and the translational rate of the *cat* gene in *E. coli* (76) but could be extended to many if not all mRNAs in the bacterial kingdom. When the environment is changing, bacteria have to constantly modulate the expression of their genes. In this dynamic adaptation process, the positive regulation of the degradation by the level of mRNA counteracts the transcription changes. Such opposite regulation is expected to fine tune the intracellular pool of mRNA, thereby avoiding fluctuations and instabilities that could be deleterious to bacterial adaptation.

To summarize, the negative relationship between mRNA concentration and stability, and particularly the stabilization of mRNAs at very low concentrations, could be a general regulatory feature to avoid complete mRNA decay over time, which could otherwise result in the incapacity of cells to resume growth, and hence lead to death. In dynamic adaptation, the transcription level of specific mRNA can be dramatically positively or negatively modified. The opposite modulation of degradation observed can therefore be considered as a way to control fluctuations in the level of mRNAs in response to stress, and to restore homeostasis rapidly in newly encountered living conditions.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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