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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 280, NO. 43, pp. 36380–36385, October 28, 2005

Role of mRNA Stability during Genome-wide Adaptation of *Lactococcus lactis* to Carbon Starvation*

Received for publication, June 2, 2005, and in revised form, August 29, 2005 Published, JBC Papers in Press, August 30, 2005, DOI 10.1074/jbc.M506006200

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The stability of mRNA was investigated for the first time at the genomic scale during carbon starvation adaptation of *Lactococcus lactis* IL1403. In exponential phase, mRNA half-lives were correlated positively to open reading frame length. A polypurine sequence, AGGAG, was identified as a putative 5′-stabilizer and inverted repeated sequences as a 3′-destabilizer. These original findings suggested that multiple pathways of mRNA degradation should coexist: internal cleavage, endonuclease cleavage initiated at the 5′-end, and exonuclease attack at the 3′-end. During carbon starvation adaptation, mRNA stability globally increased, but specific mechanisms allowing a wide range of stabilization factors between genes and differential kinetic evolution were involved. A formal method allowing the quantification of the relative influences of transcription and degradation on the mRNA pool control was developed and applied in *L. lactis*. Gene expression was mostly controlled by altered transcription prior to carbon source exhaustion, while the influence of mRNA stability increased during the starvation phase. This study highlighted that stability modulation in response to adverse growth conditions can govern gene regulation to the same extent as transcription in bacteria.

In natural ecosystems, bacteria are often submitted to stress, and cells have naturally evolved to improve their resistance to these adverse growth conditions. The response against a particular stress involves various physiological adaptations (growth, catabolism, particular metabolism, cellular morphology) and is thus pleiotropic. Recently, the development of DNA array technology allowing the quantification of mRNAs of the entire genome has enabled the global transcriptional response to be characterized for a number of bacteria during different stress conditions. Because cellular mRNA concentration depends on the relative rates of synthesis and decay, changes in the mRNA pool can occur either by transcriptional control or by modification of the susceptibility of the mRNA to degradation. However, mRNA stability has rarely been investigated at the genomic scale. Messenger half-lives have been measured in order to identify stability determinants in only two different bacteria, *Escherichia coli* and *Bacillus subtilis* (1–3). Furthermore, no study concerning the evolution of the whole genome mRNA stability during response to environmental changes can be found in the literature. Bulk mRNA fate or particular mRNA half-life changes have been investigated (4–8), but these data are not meaningful in the context of the overall physiological response. A more global study in *Lactococcus lactis* during acidic conditions was published (9), but it was restricted to a particular functional category (central metabolism). Thus, although mRNA stability can potentially participate to the expression of the genome metabolic functions, the extent to which mRNA half-life regulation contributes to the regulation of gene expression has not yet been quantitatively established.

L. lactis, a model bacterium for systems biology, is probably the most ingested bacteria by humans because of its widespread use in the food industry. This bacterium can adapt to various stresses (pH, osmotic, oxygen, nutrient starvation) that it encounters in the various environments in which it exists (natural ecosystems, industrial medium, digestive tract). One of the stress conditions receiving renewed interest is carbon starvation, because modifications of whole genome expression have been investigated for various Gram-positive bacteria such as *L. lactis*, *B. subtilis*, or *Mycobacterium tuberculosis* (10–12). However, these studies have described changes in gene expression without taking into account the possible influence of mRNA stability. In this study, mRNA half-lives were measured in *L. lactis* during both optimal growth and progressive adaptation to carbon starvation under defined physiological growth conditions. These quantitative data for mRNA stability coupled to bioinformatics analysis have enabled stability determinants to be proposed. A formal method of metabolic control analysis was developed and used to evaluate the relative influence of mRNA degradation and transcription during the global response of this bacterium to carbon starvation.

EXPERIMENTAL PROCEDURES

Organism and Growth Conditions—The bacterium used throughout this study was *L. lactis* ssp. *lactis* IL1403, whose genome was entirely sequenced (13). The strain was grown on the chemically defined CDM medium (14, 15) complemented by glucose (55 mM) as the sole carbon source. Cultures were grown under anaerobic conditions in a 2-l fermentor (Setric Génie Industriel, Toulouse, France) at a constant temperature of 30 °C and agitation speed of 250 rpm. The pH was maintained at 6.6 by automatic addition of KOH (10 N). Bacterial growth was estimated by spectrophotometric measurement at 580 nm (1 Unity of absorbance is equivalent to 0.3 $g \cdot l^{-1}$), and glucose concentrations were measured by high pressure liquid chromatography as previously described (16). Cultures were monitored from the inoculation to 4 h after glucose exhaustion. Inoculation was realized with exponential phase cells from precultures grown in the same medium so as to obtain an initial A_{580} of 0.08.

Determination of mRNA Half-lives—For mRNA half-life quantification, three growth conditions (exponential, deceleration, and carbon starvation phases) were studied in independent but physiologically identical cultures. At the required growth condition, transcription was arrested by rifampicin addition to a final concentration of 500 $\mu g \cdot ml^{-1}$. Cell samples were taken over 10 min in exponential phase or 45 min in

* This work was supported by a grant from the Toulouse Génopole. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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deceleration and starvation phases. Four different time points, including the reference sample (before rifampicin addition), were analyzed simultaneously as described below (RNA extraction, cDNA preparation, hybridization, and detection). At least three independent time courses were analyzed for each condition. mRNA half-lives ($t_{1/2}$) were calculated from the degradation rate constant (k) corresponding to the slope of a semi-logarithmic plot of mRNA amount as a function of time with the relation $t_{1/2} = \ln 2/k$. Raw data of mRNA half-lives were filtered with a minimum regression coefficient of 0.7 and a limit of precision in the repetitions corresponding to the mean value of standard deviation (40% in the exponential phase and 60% in the deceleration and starvation phases). The complete lists of half-lives calculated during the three phases are available at biopuce.insa-toulouse.fr/supdata/rna-decay-2005/.

RNA Extraction—A volume of culture corresponding to 6 mg (dry weight) of cells was frozen immediately in liquid nitrogen. Before cell lysis, each sample was centrifuged (4 °C, 5 min, 8000 rpm), washed with 1 ml of TE buffer (Tris-HCl 10 mM, pH 8, EDTA 1 mM) and resuspended in 500 μl of TE buffer. Cells were disrupted at 4 °C (3 cycles of 1 min interspaced by 3-min cooling periods) on a minibead beater (Biospec Products) with glass beads (0.6 g), 50 μl of SDS (10%), and 500 μl of phenol (pH 4.7). After centrifugation to eliminate cell debris and phenol (4 °C, 25 min, 13,000 rpm), half of the aqueous phase containing RNA was extracted with a RNeasy midi kit (Qiagen), including the DNase I treatment described in the manufacturer's instructions. RNA was quantified at 260 and 280 nm, and RNA quality was controlled on electrophoresis-agarose gel in denaturing conditions.

cDNA Preparation—For cDNA synthesis, 10 or 15 μg of total RNA was mixed with 1 μl of random hexamer primers (500 ng·μl^{-1}; Sigma Genosys), 1 μl of Eurogentec *L. lactis* open reading frame-specific primers (500 ng·μl^{-1}), and sterile water to a final volume of 24 μl. The mixture was heated at 70 °C for 5 min and then immediately cooled to 4 °C. Reverse transcription was performed for 1 h at 42 °C in a total volume of 50 μl, with SuperScriptII reverse transcriptase (300 units; Invitrogen), dithiothreitol (10 mM; Invitrogen), dATP, dGTP, dTTP (0.3 mM each), and [α-^{33}P]dCTP (50 μCi; Amersham Biosciences) and 5× first strand buffer (1×; Invitrogen). After 1 h, SuperScriptII reverse transcriptase (100 units) and dCTP (0.1 mM) were added and the reaction performed for one more hour at 42 °C. Reaction was stopped by heating (15 min at 70 °C), and the remaining RNA was hydrolyzed by RNase H (2 units, 20 min, 37 °C; Invitrogen). Before hybridization, labeled cDNA was purified on Microspin G25 columns (Amersham Biosciences) according to the manufacturer's instructions.

Hybridization and Detection—*L. lactis* IL1403-specific PCR products were spotted in duplicate on positively charged nylon membranes (4 deposits/spot; Plateforme Génomique, Toulouse). A single PCR/open reading frame (ORF)[2] was designed by Eurogentec. PCRs covered ~71% of each ORF length (mean length 535 bp and final concentration between 40 and 180 μg·ml^{-1}). 2053 ORFs of the 2310 identified on the genome were effectively available. Prior to hybridization, membranes were washed for 5 min in 50 ml of 2× SSPE (SSPE 1× is 0.18 mM NaCl, 1 mM EDTA, and 10 mM phosphate buffer, pH 7.2) and prehybridized for 3 h at 68 °C in 5 ml of hybridization buffer (5× SSPE, 2% SDS, 1× Denhardt's reagent). Labeled cDNA was heated for 10 min at 95 °C and immediately cooled at 4 °C for 5 min. Membrane hybridization was carried out for 15 h at 68 °C with 5 ml of hybridization buffer containing labeled and denatured cDNA. Membranes were then washed three times in 50 ml of washing solution (0.5× SSPE, 0.2% SDS) for 5 min at room temperature and three times in 50 ml of preheated washing solution for 20 min at 68 °C. Dried membranes were exposed to a phosphorimaging screen for 3 days and scanned with a phosphofluoroimager (Storm 860; Amersham Biosciences). Hybridization signals were quantified and assigned to gene names with the Bioplot software (developed by S. Sokol in Plateforme Génomique, Toulouse). After background removal, spots with intensities below the detection limit, defined as the mean intensity of "empty" spots, were removed.

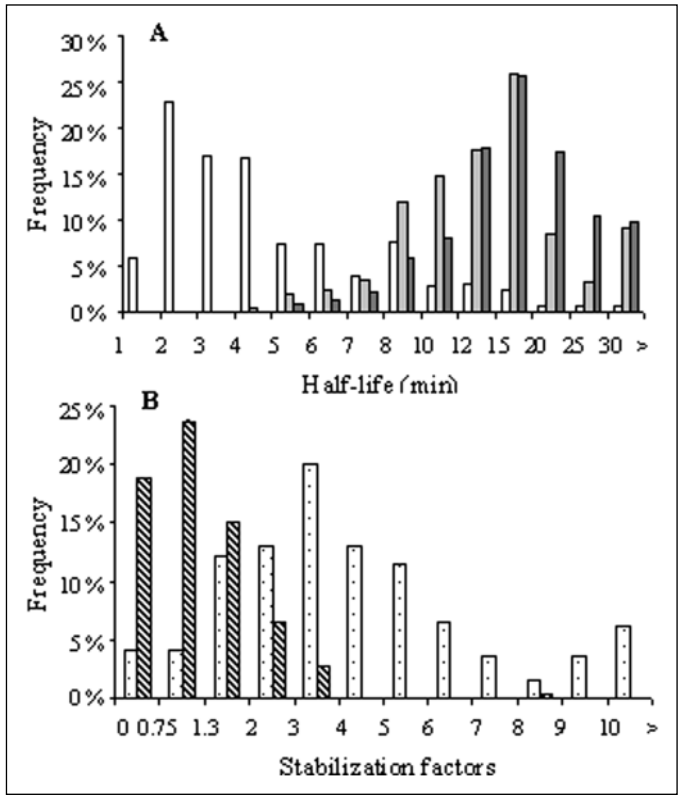

FIGURE 1. *A*, half-life frequencies distribution in exponential (*open column*), deceleration (*light gray*), and starvation (*dark gray*) phases during carbon starvation adaptation of *L. lactis*. *B*, stabilization factor frequency distribution between exponential and deceleration phases (*dotted column*) and between deceleration and starvation phases (*hatched column*) during carbon starvation adaptation of *L. lactis*.

mRNA Concentration Calculation—mRNA concentrations were used both to analyze the stability determinants of mRNA half-lives and to quantify the influence of stability control on gene expression. The three growth conditions (exponential, deceleration, and carbon starvation phases) were previously characterized from the transcriptional viewpoint (12), but values were recalculated to obtain mRNA concentrations rather than abundances. Raw data were standardized by the all spots' mean intensity of the exponential phase and corrected by total RNA concentration in each growth condition (11.7 ± 1.3, 7.6 ± 0.8, and 8.2 ± 1.4 g (100 g dried cells^{-1}) in exponential, deceleration, and starvation phases respectively).

RESULTS

Stability Determinants in Exponential Growth—mRNA stability was examined with DNA arrays during the exponential growth of *L. lactis* on glucose CDM medium, and 817 different mRNA half-lives were quantified simultaneously. mRNA half-lives of genes *adhE*, *ccpA*, and *tpi* were confirmed with Northern blot analysis (data not shown) and previous data obtained with fluorescent labeling (17). Though the large majority of half-lives (90%) ranged from 1.4 to 10 min, data were scattered between 1.4 and 74 min, (Fig. 1*A*), and the mean half-life was

[2] The abbreviations used are: ORF, open reading frame; UTR, untranslated region.

TABLE ONE

mRNA half-life determination during carbon starvation adaptation of *L. lactis*

	Exponential phase	Deceleration phase	Starvation phase
Mean half-time (min)	5.8 ± 5.6	17.6 ± 13.0	19.4 ± 10.0
Median half-time (min)	4.2	14.4	17.3

affected by a strong standard deviation (5.8 ± 5.6, TABLE ONE). To better understand this half-life disparity, correlations between mRNA half-lives and various transcript features were investigated. Pearson correlation coefficient (r) was considered statistically significant when associated p values obtained by Student's t test were lower than 10^{-3}. An inverse relationship between log-transformed mRNA half-life and ORF length ($p = 2{\cdot}10^{-4}$, $r = -0.13$) was observed. Neither study of particular messenger stability (18, 19) nor genome-wide analysis of mRNA half-life (1) had allowed such a relationship to be established with other organisms, so this may be specific to *L. lactis*. Transcript degradation rate is generally considered to be limited by endonucleolytic cleavage of the messenger (20–22). This step is achieved in *E. coli* by RNase E that preferentially cleaves single-stranded A+U-rich regions (23). However, in *L. lactis* no significant correlation was observed between mRNA half-lives and A+U content in ORFs. Most of the discrete stability determinants previously identified have been located in 5′- and 3′-untranslated regions (UTRs) (18, 22, 24, 25). The UTRs, not formally identified in *L. lactis* because its genome was not entirely annotated, were defined by 100-bp non-ORF overlapping upstream and downstream sequences in agreement with the 84- and 79-bp mean length of *E. coli* 5′- and 3′-UTRs (1). To identify stabilizing or destabilizing determinants in these presumed UTRs, an RSA-tools interface was used with a background model (26) and genes were discriminated by stability quartiles. For the genes of the fourth quartile (stable messengers, $t_{1/2} > 6.7$ min), an over-representation in the 5′-region of the purine-rich sequence AGGAG was highlighted and postulated to confer mRNA stability. Similarly, over-representation of two inverted repeated sequences, CTGTCAGTA and TACTGACAG, was observed in downstream sequences of first quartile genes (unstable messengers, $t_{1/2} < 2.8$ min), suggesting a destabilizing effect for this feature.

Because intracellular mRNA concentrations depend on the degradation rate, a comparison of messenger half-lives with their respective concentrations was carried out. mRNA concentrations of 2032 genes were quantified during exponential phase of *L. lactis* from the data of Redon *et al.* (12) as indicated under "Experimental Procedures." A significant inverse relationship between messenger half-lives and their respective concentrations ($p = 3{\cdot}10^{-4}$, $r = -0.13$) was obtained after log transformation, indicating that *L. lactis* genes with highest expression were less stable. This finding was surprising because highly expressed messengers were usually considered to be stable (24). However, such an inverse correlation has already been observed in *E. coli* by whole genome analysis (1), suggesting that mRNA half-lives are not general predictors of messenger expression.

Despite the fact that mRNA stability has often been suggested to be involved in cellular physiology, relationship between gene function and mRNA half-life has only rarely been formally demonstrated. Thus, mRNA half-life data were stratified using the functional classification described by Bolotin *et al.* (13), and a one-way analysis of variance led us to identify significant differences in mean half-lives ($p < 10^{-4}$) between metabolic functional categories. To evaluate the relative independence of the different functional categories, multiple Student's t tests were carried out (TABLE TWO). This approach revealed that seven functional categories (amino acid biosynthesis; cell envelope; cellular processes; fatty acid and phospholipid; purines, pyrimidines, nucleosides and nucleotides metabolism; regulatory functions; transport and binding proteins) exhibited significantly lower half-lives. Inversely, translation and the category named "other" (*i.e.* essentially phage- and prophage-related function genes) presented significantly higher half-lives.

TABLE TWO

Mean mRNA half-life by functional categories during exponential growth of *L. lactis*

Functional category[a]	Mean half-life
	min
Cellular process	3.8[b]
Regulatory functions	3.9[b]
Fatty acid and phospholipid metabolism	4.0[b]
Amino acid biosynthesis	4.2[b]
Cell envelope	4.3[b]
Cofactors, prosthetic groups, carriers biosynthesis	4.6[c]
Transport and binding proteins	5.0[b]
Purines, pyrimidines, nucleosides, and nucleotides	4.0[b]
Replication	4.7[c]
Unknown function	5.7[c]
Energy metabolism	6.1[c]
Transcription	8.6[c]
Central intermediary metabolism	10.3[c]
Translation	7.0[d]
Other	10.9[d]

[a] Functional categories defined by Bolotin *et al*. (13).
[b] Mean half-life significantly shorter ($p < 0.05$).
[c] Mean half-life not significantly different.
[d] Mean half-life significantly longer ($p < 0.05$).

Stability and Carbon Starvation Adaptation—The fate of whole genome messengers was investigated in *L. lactis* during progressive carbon starvation. As for previous experiments (12), the growth was exponential during the first 5 h of culture and stationary phase was reached when glucose was exhausted at 6 h of culture. Between 5 and 6 h of fermentation, growth rate decelerated with the progressive decrease of glucose concentration from a threshold level of 14 mM until zero. The mRNA stability was investigated at the genomic scale in transitional and stationary phases, and 452 and 579 messenger half-lives were measured, respectively (Fig. 1*A*). mRNA concentrations were also determined in each phase from the data of Redon *et al.* (12), showing a general decreasing profile except for a minority of genes (1.6%). No correlation was found between the half-lives of mRNA observed in exponential phase and those measured in deceleration or starvation phases, suggesting that different mechanisms were controlling stability in the last two phases. Unlike in the exponential phase, no link between mRNA half-lives and functional categories was observed in either deceleration or starvation phases. In the deceleration phase, mRNA half-lives did not exhibit any significant correlation with ORF length or mRNA concentrations, and no predominant pattern was identified in 5′- or 3′-regions whatever the level of stability concerned. However, the stability pattern differed significantly during the starvation phase, because mRNA half-lives were found to be positively correlated to mRNA concentrations (log-transformed data, $p = 2{\cdot}10^{-11}$, $r = 0.28$). It should be noticed that the relationship between expression levels and mRNA half-lives was inverted compared with that seen in the exponential phase. Furthermore, the relationship described for the exponential phase cells between the highest messenger half-lives and the significant over-representation

of the polypurine sequence AGGAG in upstream sequences (5′-region) was restored.

An important increase of median half-lives in the deceleration and starvation phases (14.4 and 17.3 min, respectively) compared with exponential phase values was observed (TABLE ONE), indicating that mRNAs were globally stabilized in response to carbon starvation. Efficient and sensitive sensors should be involved in this mRNA decay phenomena, because the stabilization occurred before glucose exhaustion ([glucose] <14 mM). Stabilization factors, expressed as a ratio of half-lives, were calculated for each mRNA between the exponential and the deceleration phases and between the deceleration and stationary phases. As shown in Fig. 1*B*, the statistical partition of these ratios differed, and median ratio between exponential and deceleration phases was much higher than between deceleration and stationary phases (3.9 and 1.1, respectively). Taking into account the precision in stabilization factor determination, it was estimated that 92% of transcripts were stabilized during deceleration phase in comparison to exponential phase. The evolution of mRNA half-lives was more contrasted between deceleration and starvation phases, because 37% of messengers were further stabilized while 28% were destabilized. To get a more precise insight into this stabilization, features controlling stabilization factors were investigated. Stabilization factors were not linked to ORF length or base composition or functional categories. However, a significant negative correlation was observed between deceleration/exponential stabilization factors and exponential half-lives ($p = 5{\cdot}10^{-6}$, $r = -0.29$), indicating that the unstable messengers in exponential phase were those most stabilized during the transition phase, and inversely. This preferential stabilization of the most unstable messengers was confirmed between deceleration and starvation phases ($p = 1{\cdot}10^{-12}$, $r = -0.52$).

mRNA Pool Regulation Analysis—Changes in mRNA concentrations in the cells can be achieved by changes in transcriptional and/or degradation rates. To quantify the relative importance of the two modes of regulation during carbon starvation adaptation, the approach developed by ter Kuile and Westerhoff (27) and adapted by Even *et al.* (28) was used. At steady state, mRNA concentration can be expressed as a function of transcription rate V_T and degradation constant rate k ($V_T = k{\cdot}$[mRNA] with $k = \ln 2/ t_{1/2}$). Dilution rate of the messengers due to cellular growth was neglected because generation time was in general significantly lower than the mRNA half-lives. Assuming that V_T and k were independent, degradation (ρ_D) and transcription (ρ_T) regulation coefficients were defined as shown in Equation 1.

$$\rho_D = -\frac{d\ln k}{d\ln[\mathrm{mRNA}]}, \rho_T = \frac{d\ln V_T}{d\ln[\mathrm{mRNA}]}, \rho_D + \rho_T = 1 \qquad \text{(Eq. 1)}$$

The degradation regulation coefficient ρ_D was calculated as the opposite slope of the double logarithmic plot of degradation rate k *versus* mRNA concentration between exponential and deceleration phases and between deceleration and starvation phases. Between exponential and deceleration phases 92% of genes exhibited low ρ_D values ($\rho_D < 0$), indicating that mRNA concentrations were not controlled by degradation but mostly by transcription (Fig. 2). Between deceleration and starvation phases, the influence of the two modes of regulation was more balanced (Fig. 2). Indeed, mRNA concentration was still controlled at the transcriptional level for 51% of genes ($\rho_D < 0$), whereas 25% of genes were controlled by degradation ($\rho_D > 1$) and 24% presented a shared control ($0 < \rho_D < 1$), indicating that mRNA stability plays a significant, if not overriding, effect on modulating the adaptation of this bacterium to carbon starvation.

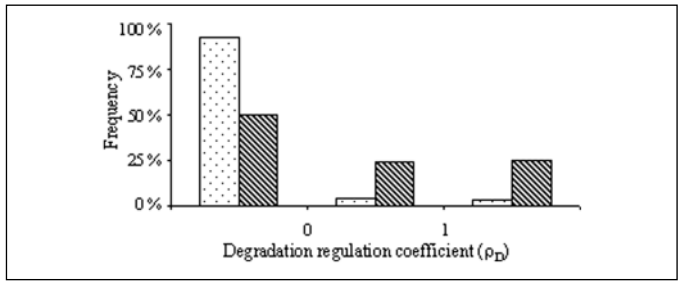

FIGURE 2. **Frequency of genes exhibiting regulation coefficient ρ_D inferior to 0 (transcriptional control), between 0 and 1 (shared control), and superior to 1 (degradation control) between exponential and deceleration phases (*dotted column*) and between deceleration and starvation phases (*hatched column*) during carbon starvation adaptation of *L. lactis*.**

DISCUSSION

mRNA Degradation Mechanisms—This study provided the first characterization of whole genome mRNA decay in *L. lactis* and more generally in a lactic acid bacterium. Messenger half-lives were measured for more than 35% of genes, thus providing a general overview of mRNA stability. As observed in *E. coli* (1) or *B. subtilis* (2), messenger RNAs exhibited short but wide ranged half-lives, revealing important variations of decay rate in the cell. mRNA stability seemed strongly linked to physiological gene functions, at least during exponential growth phase, confirming that mRNA stability should follow a functional logic, as also suggested by global studies in *E. coli* (1, 3) and *Saccharomyces cerevisiae* (29). As mRNA half-life defines the time a messenger is accessible for translation, housekeeping genes like ribosomal protein-encoding genes displayed high stability. Inversely, genes whose expression should be rapidly adjusted to environmental growth conditions, like regulatory or transport-encoding genes, presented the lowest stability. Surprisingly, messenger half-lives were shown to be linked to ORF length. This feature, not previously observed in microorganisms, suggested that mRNA decay might be related to broad specific endonucleolytic cleavage at internal sites evenly distributed along the messenger. As stability was not related to A+U content, these sites should differ from the AU-rich sites specific of RNase E cleavage in *E. coli* (23). To date, the lack of information concerning the endonuclease activity involved in messenger degradation in *L. lactis* and more generally in Gram+ bacteria (22, 30) is limiting the mechanistic understanding of mRNA decay. However, the over-representation of the purine-rich sequence AGGAG in the 5′-region of stable messengers implied that the 5′-end may be involved in the control of mRNA degradation. This sequence, previously identified in the *Bacillus thuringiensis cryIIIA* gene (31) and in a segment of RNA from *B. subtilis* bacteriophage SP82 (32), was complementary to the 3′-end of 16 S rRNA and is assumed to confer mRNA stability by impeding endonuclease activity due to ribosome pausing. The stabilizing effect of such a polypurine sequence identified for the first time for a broad range of mRNAs could indicate that ribosome binding to the 5′-end may occupy a more general role in stability control than presently believed. Finally, inverted repeated sequences with potential for forming stem-loop structures in the 3′-region were shown in this study to be associated with mRNA instability for a broad range of genes. Inverted repeated sequences, such as repetitive extragenic palindromic (REP) sequences, have been usually considered to stabilize messengers by impending 3′- 5′-exonuclease attack at the 3′-end (33, 34). However, REP sequence from *cat* mRNA was shown to destabilize *ompA* fusions in *E. coli* when inserted into its 3′-end (35), showing that REP sequences are not necessarily involved in stabilization processes. The destabilization mechanism related to inverted repeat sequences could involve the double-stranded specific ribonuclease RNase III iden-

tified in *E. coli* or *B. subtilis* (22, 36) but also in *L. lactis* (37, 38), by generating, after cleavage of the stem-loop structures, a new 3′-end particularly sensitive to exonucleases.

The identification of a 5′-stabilizer suggests that mRNA decay is controlled by the initiation of degradation at the 5′-end as postulated in *E. coli* and *B. subtilis* (19 ,22,39). However, the assumption of internal cleavage along the messenger and the identification of a 3′-destabilizer both indicate that multiple pathways of degradation should coexist in *L. lactis*.

Modulation of Stability in Response to Carbon Starvation—Investigating mRNA half-life in *L. lactis* allowed us to highlight that a 4-fold stabilization occurred in response to carbon starvation as indicated by the increase of median half-lives (TABLE ONE). Increase of bulk mRNA half-life has previously been reported in *E. coli* and *Vibrio* S14 (4, 5), suggesting that stabilization mechanism during carbon starvation could be widespread in bacteria. Stability increases in response to environmental conditions have been described for various discrete messengers (for a review see Ref. 6), but this study provided the first evidence that a genome-scale stabilization occurs during carbon starvation. However, the wide range of stabilization factors, the differential stability trends (*i.e.* higher stabilization for unstable messengers), and the kinetic evolution of mRNA stability (*i.e.* general stabilization in deceleration phase followed by a differential stabilization in starvation) revealed that specific regulations are involved in the general control of mRNA stability. Different mechanisms, such as negative control of the degradation machinery or mRNA interactions with proteins (like Hfq or CsrA) or non-coding RNAs (for reviews see Refs. 40, 41), might be involved in these general and/or specific stability phenomena. However, ribosome protection in the 5′-region should actively participate in these stabilization mechanisms. Indeed, when exponential and carbon starvation phases were compared, the decrease of total RNA concentration and the proportion of mRNA in this total RNA (12) indicated that the rRNA/mRNA ratio increased 2.3-fold during carbon starvation in *L. lactis*. The frequency of bound ribosomes in the 5′-region may thus increase and impede the initiation of degradation by endonuclease as previously proposed in *B. subtilis* (42, 43). Furthermore, a denser ribosome binding on messenger should inhibit degradation by masking endonuclease internal cleavage sites, as postulated in *E. coli* (44, 45).

The impact of mRNA degradation on the adaptation of *L. lactis* to carbon starvation was quantitatively evaluated in this work, comparing the respective influence of degradation and transcription rates on mRNA pool alterations. The early adaptation in deceleration phase was mostly controlled by transcription and not by stability, though high stabilization occurred in this phase. Because transcription rate sharply decreases (12), predominant control of the transcription probably accounted for the inverse relationship observed between mRNA concentrations and half-lives. The general stabilization obtained in this early adaptation allowed mRNA pool exhaustion to be limited despite the drastic reduction in the transcriptional rate. Fine gene-specific regulations directed further adaptation, when cells effectively encountered starvation. Though transcription still remained the major phenomena for 51% of the genes, degradation control became more influential because for 25% of the genes degradation was the main mode of mRNA pool regulation between deceleration and starvation phases (the remainder responding to both phenomena). Such a high influence of degradation has been demonstrated previously in the control of the abundance of ribosome biogenesis factors (46). The higher influence of stability regulation in the starvation phase is in agreement with the restoration of a positive correlation between concentrations and mRNA half-lives, linking the most stable to the most expressed mRNAs. In *L. lactis*, sugar catabolism is the main source of biological energy; therefore, such stabilization in a general context of diminished transcription rates should be crucial to reduce energy consumption during carbon starvation. It would be interesting to determine whether gene expression is controlled with such repartition between transcription and degradation during stress or nutrient starvation other than by the energy source.

This control analysis at the genomic scale formally demonstrated that mRNA stability control is a significant part of the gene expression response to adverse conditions, alongside the more classically studied transcriptional regulation. Therefore, modulation of gene expression is not necessarily linked only to transcriptional regulations. As post-genomic investigation tools are becoming widely available, it is necessary to take into account effects such as mRNA stability if a functional understanding of global cellular regulation is to be achieved.

Acknowledgment—We thank Nic Lindley for useful discussions and for careful proofreading of the manuscript.

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