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Measurements of mRNA Degradation in *Borrelia burgdorferi*

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The importance of gene regulation in the enzootic cycle of *Borrelia burgdorferi*, the spirochete that causes Lyme disease, is well established. *B. burgdorferi* regulates gene expression in response to changes in environmental stimuli associated with changing hosts. In this study, we monitored mRNA decay in *B. burgdorferi* following transcriptional arrest with actinomycin D. The time-dependent decay of transcripts encoding RNA polymerase subunits (*rpoA* and *rpoS*), ribosomal proteins (*rpsD*, *rpsK*, *rpsM*, *rplQ*, and *rpsO*), a nuclease (*pnp*), outer surface lipoproteins (*ospA* and *ospC*), and a flagellar protein (*flaB*) have different profiles and indicate half-lives ranging from approximately 1 min to more than 45 min in cells cultured at 35°C. Our results provide a first step in characterizing mRNA decay in *B. burgdorferi* and in investigating its role in gene expression and regulation.

Lyme disease is caused by the bacterium *Borrelia burgdorferi*, a spirochete that cycles between *Ixodes* ticks and vertebrate hosts in nature. *B. burgdorferi* is acquired by a larval tick when the tick feeds on an infected vertebrate host. After acquiring the bacteria, the larval tick will remain infected as it molts to the nymphal and adult stages, and it can transmit *B. burgdorferi* during subsequent blood meals. Tick feeding initiates movement of *B. burgdorferi* from the tick midgut to the salivary glands and, ultimately, into the new vertebrate host (reviewed in reference 1). The incidence of Lyme disease in the United States has increased dramatically in the last decade, with 30,158 cases reported in 2010 (2).

Successful transmission from the tick vector to the vertebrate host depends on *B. burgdorferi*'s ability to survive the transition between two very distinct environments, which requires a dramatic shift in gene expression pattern in this pathogen (reviewed in references 1 and 3). To better understand the life cycle of this spirochete and its virulence, much of the work on gene regulation in *B. burgdorferi* has been aimed at understanding how specific genes are affected by the environmental signals associated with switching hosts. mRNA levels are affected by temperature (215 genes) (4), tick feeding (24 genes) (5), and mammalian host factors (125 genes) (6). The alternative sigma factor RpoS is induced upon tick feeding (5) and is required for mammalian infection (7). Artificial induction of *rpoS* results in changes in transcript levels of 137 genes (8). Controlling gene expression is essential for *B. burgdorferi* to complete its enzootic life cycle. Regulation of mRNA levels in response to changes in the environmental stimuli associated with changing hosts depends on controlling transcription and is likely to depend upon the ability of the cell to degrade mRNA in a timely fashion. mRNA degradation in other bacteria is extensively regulated (reviewed in reference 9–13); however, mRNA degradation pathways have not yet been characterized in any *Borrelia* species.

B. burgdorferi is a slow-growing bacterium with generation times between 8 and 12 h at temperatures between 33 and 37°C in liquid media (14). mRNA decay rates in other bacterial species generally appear to be independent of doubling time (reviewed in reference 15). The average lifetime of mRNA in other bacteria is typically on the order of a few minutes (reviewed in reference 15); however, *B. burgdorferi* may have some extremely stable transcripts. In *B. burgdorferi* cultures killed by the antibiotic ceftriaxone, mRNA fragments are detectable for 14 days posttreatment, 11 days after the cells can be successfully revived and subcultured (16).

Phylogenetic analysis (Table 1) (17, 18) suggests that this spirochete has only a subset of the nucleases involved in mRNA degradation that are involved in mRNA decay pathways in either *Bacillus subtilis* or *Escherichia coli* (for recent reviews, see references 12, 13, 15, and 19–21). The limited subset of ribonucleases present in *B. burgdorferi* may influence degradation pathways and the persistence of mRNA transcripts.

B. burgdorferi is naturally resistant to rifampin, the antibiotic that is used to arrest transcription in most studies of prokaryotic mRNA decay. Rifampin interacts with the beta subunit of RNA polymerase in the RNA exit channel, preventing transcript growth beyond 2 or 3 nucleotides (22). Schwartz and coworkers first determined that the sequence of the *B. burgdorferi rpoB* gene differs from *E. coli*'s wild-type sequence at positions that are hot spots for rifampin-resistant mutations in *E. coli* and *Mycobacterium tuberculosis* (amino acid positions 500, 508, 518, 522, 531, 539, and 552 using *E. coli* numbering) (23).

In this study, we used actinomycin D as a transcription inhibitor to enable measurement of mRNA half-life ($t_{1/2}$) in *B. burgdorferi*. The polypeptide antibiotic actinomycin D was used in 1962 to study mRNA turnover in *B. subtilis* (24) and is currently used extensively to arrest transcription in eukaryotes and archaea (25–28). We measured mRNA half-lives at 35°C for a variety of genes in *B. burgdorferi*, including genes that are differentially expressed during the enzootic cycle. These studies are the first to examine mRNA decay in *B. burgdorferi* and demonstrate diverse mRNA decay profiles. Our studies are a first step in characterizing mRNA decay mechanisms in this important pathogen.

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TABLE 1 Comparison of ribonucleases found in *Escherichia coli*, *Bacillus subtilis*, and *Borrelia burgdorferi*[a]

Nuclease	Presence in:			Locus
	E. coli	*B. subtilis*	*B. burgdorferi*	
RNase E or G	+	−	−	−
RNase III	+	+	+	BB0705
RNase II	+	−	−	−
RNase M5	−	+	+	BB0626
RNase Y	−	+	+	BB0504
RNase J1	−	+	+/−	BB0533
RNase J2	−	+	−	−
RNase Z	+	+	+	BB0755

[a] The plus symbol indicates that a gene was previously annotated (18) and/or identified through performing a translated nucleotide database search using an *E. coli* K-12 (57) or *B. subtilis* 168 (58) query in tBLASTn (59), performed at the Comprehensive Microbial Resource website (60). The minus symbol indicates that no homolog was detected (RNase E, RNase II, and RNase J2). *B. subtilis* RNase J1 (555 amino acids) and *B. burgdorferi* B31 PhnP (762 amino acids) share homology of 124 amino acids (BLAST score, 73; identities, 31/124; positives, 54/124), indicated by +/−.

MATERIALS AND METHODS

***Borrelia burgdorferi* strains and culture conditions.** *B. burgdorferi* strains A3-LS-*flacp-ospC* (29) and 297-LK-*flacp-rpoS* (8), in which either the *ospC* or *rpoS* gene, respectively, is under the control of an inducible *lac* promoter system, were a kind gift of Michael Gilbert. *B. burgdorferi* B31A, a high-passage strain, was the kind gift of Scott Samuels and Laura Hall. *B. burgdorferi* was grown at 35°C and 5% CO_2 in Barbour-Stoenner-Kelley II (BSK-II) complete medium (in 6% rabbit serum) (Pel Freeze; Rogers, AR) (30) supplemented with the strain-appropriate antibiotics: A3-LS-flacp-ospC with 200 μg ml^{-1} kanamycin (Fisher Scientific, Fairlawn, NJ) and 50 μg ml^{-1} streptomycin (Fisher Scientific, Fairlawn, NJ), 297-LK-*flacp-rpoS* with 200 μg ml^{-1} kanamycin and 40 μg ml^{-1} gentamicin (Fisher Scientific, Fairlawn, NJ), and B31A with 25 μg ml^{-1} rifampin (Sigma-Aldrich, St. Louis, MO). Rifampin (Sigma-Aldrich, St. Louis, MO) was used with B31A cultures to prevent contamination by other bacteria. When used, the actinomycin D (TOCRIS Bioscience, Bristol, United Kingdom) concentration in cultures was 150 μg ml^{-1}, and isopropyl β-D-1-thiogalactopyranoside (IPTG) (Fisher Scientific, Fairlawn, NJ) was added at 1 mM. Cultures were grown to mid-log phase (1×10^7 to 7×10^7 cells ml^{-1}) before the start of each experiment (e.g., addition of actinomycin D or IPTG). Cell densities were determined using a Petroff-Hausser counting chamber under dark-field microscopy.

RNA isolation. For RNA isolation, aliquots of 25 to 30 ml of *B. burgdorferi* culture, containing between 2.75×10^8 and 8.25×10^9 cells, were centrifuged at 4°C and 5,000 × *g* for 10 min, resuspended in 700 μl RLT buffer (Qiagen, Valencia, CA), placed in sterile 2-ml screw-cap tubes with approximately 0.25-ml sterile zirconia beads, and then subjected to shaking at 30 Hz for 5 min in a Qiagen tissue lyser II. RNA was purified using an RNeasy mini kit according to the manufacturer's protocol (Qiagen, Valencia, CA). RNA samples were treated with Turbo DNA-free DNase I (Applied Biosystems/Life Technologies, Grand Island, NY) by following the manufacturer's protocol. RNA concentration and quality were determined spectrophotometrically. Samples were diluted in nuclease-free water to a standard concentration of total RNA (usually 20 ng μl^{-1}) and were stored at −20°C.

qRT-PCR. RNA, purified form *B. burgdorferi*, was quantified with one-step reverse transcription and real-time quantitative PCR (qRT-PCR) using the EXPRESS one-step SYBR green ER kit (Invitrogen, Carlsbad, CA) on a Stratagene MX3000P thermocycler (Agilent Technologies, Inc., Wilmington, DE) using primers that amplify our genes of interest (see Table S1 in the supplemental material). qRT-PCRs were carried out according to the manufacturer's protocol using 40 ng total RNA per 20-μl reaction mixture unless otherwise specified. The absence of contaminating DNA was confirmed by performing PCR in the absence of reverse transcriptase. Cycle threshold (C_T) values are the number of cycles required during amplification for the fluorescent signal to exceed background fluorescence levels and were obtained using the default settings in MxPro-Mx200P v4.10 software (Agilent Technologies, Inc., Wilmington, DE) in the Stratagene Mx3000P thermocycler. Efficiencies of the qRT-PCR for each primer set were determined using total RNA in a dilution series across two orders of magnitude. Efficiencies ranged from 87 to 120%.

Data analysis. To determine the fraction of mRNA for a gene of interest that remains following antibiotic treatment, posttreatment C_T values were compared to reference C_T values (i.e., $C_{T\mathrm{ref}}$) for the same transcript for RNA purified from the same cultures prior to antibiotic addition or from a control culture grown without antibiotic treatment. The fraction of remaining RNA (f) was measured as $f = 2^{(CT\mathrm{ref} - CT)}$, where $C_{T\mathrm{ref}}$ is the C_T value determined for the mRNA from an antibiotic-free culture and C_T is the value for the mRNA purified from culture at a given time after antibiotic addition. To determine the rate constant and half-life for each mRNA, the fraction of remaining RNA was plotted against time points from 0 to 45 min, and the data were globally fit using Sigma Plot 12.0 (Systat Software Inc.) to an equation for first-order decay, $f = f_0(e^{-kt})$, in which k is the first-order rate constant, f_0 is the initial fraction of RNA, and t is time. Each rate constant was determined from a simultaneous global fit of data for all biological replicates and qRT-PCR replicates for each transcript. These fits assume that the rate constants are independent of the experimental variability present in the determination of $C_{T\mathrm{ref}}$ from individual samples.

RESULTS

To develop a method for studying mRNA decay in rifampin-resistant *B. burgdorferi*, we tested the effectiveness of actinomycin D in arresting transcription. We initially attempted to examine the effects of actinomycin D on total RNA synthesis by comparing levels of [^{3}H]uridine incorporation in the presence and absence of actinomycin D (for example, see reference 31), but we observed very low levels of incorporation of [^{3}H]uridine under all growth conditions (data not shown), preventing a direct measurement of the effect of actinomycin D on total RNA synthesis. We then tested whether actinomycin D could prevent induction of *ospC* from the IPTG-inducible *flac* promoter in A3-LS-*flacp-ospC* cells (29) that were cultured at 35°C. In these experiments, mid-log-phase cultures were treated with actinomycin D for 30 min prior to the addition of IPTG. *B. burgdorferi* cell density did not increase significantly following addition of actinomycin D, although cells retained motility for several hours after exposure at 35°C (data not shown). Three hours following actinomycin D treatment, RNA was purified from cultures, and *ospC* and *flaB* transcript levels were assayed by qRT-PCR using gene-specific primers (see Table S1 in the supplemental material). The amplification curves (Fig. 1) indicate that the fluorescence signal increases with the number of amplification cycles as expected. The amplification curve for *ospC* from cultures that lack actinomycin D and contain IPTG (Fig. 1A, open circles) are significantly shifted to a lower number of cycles relative to those obtained from control cultures lacking both actinomycin D and IPTG (closed circles), indicating that addition of IPTG increases *ospC* mRNA levels, as expected by transcriptional induction. For cultures treated with actinomycin D, there is little difference in the transcript levels resulting from the addition of IPTG (Fig. 1A, triangles), indicating that actinomycin D prevents induction of transcription of *ospC* from the *flac* promoter. In this strain, *flaB* is controlled by its natural promoter, which is not induced in the presence of IPTG. As expected, the *flaB* transcripts show no significant effect caused by the addition of IPTG for cultures where actinomycin D is present or absent (Fig. 1B).

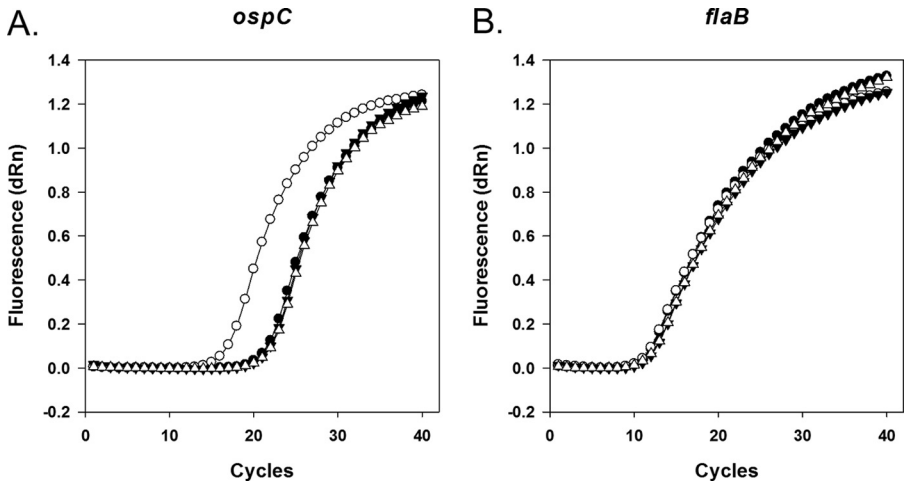

FIG 1 Actinomycin D prevents induction of transcription of *ospC* controlled by an IPTG-inducible promoter. Quantitative RT-PCR amplification curves are shown for *ospC* (A) and *flaB* (B) transcripts. A3-LS-*flacp-ospC* cultures at mid-log growth phase were split, and half of the initial culture was treated with actinomycin D (final concentration, 150 μg ml^{-1}). Thirty minutes later, cultures were split again and treated with IPTG or used as controls lacking an inducer. Cells were cultured for an additional 3 h before RNA isolation and qRT-PCR amplification. ●, Lacking both actinomycin D and IPTG ($C_{T\ ospC}$ = 22.22; $C_{T\ flaB}$ = 12.42); ○, lacking actinomycin D but containing IPTG ($C_{T\ ospC}$ = 17.51; $C_{T\ flaB}$ = 12.30); ▼, containing actinomycin D but lacking IPTG ($C_{T\ ospC}$ = 22.57; $C_{T\ flaB}$ = 12.96); Δ, containing both actinomycin D and IPTG ($C_{T\ ospC}$ = 22.70; $C_{T\ flaB}$ = 12.87). Ten ng of total RNA was used in each reaction mixture. dRn, magnitude of the fluorescence signal over background fluorescence generated at each time point.

To assess whether we could measure mRNA degradation by arresting transcription with actinomycin D, we used qRT-PCR to compare *rpoS* mRNA degradation from an inducible strain (297-LK-*flacp-rpoS*) (8) when transcription was arrested by removing inducer (analogous to experiments performed in *E. coli* [32]) to that measured after actinomycin D addition. Decreasing mRNA levels will result in a higher number of amplification cycles to reach the threshold cycle (C_T) where the fluorescent signal resulting from the amplification of the RNA is higher than that observed from the background. A complete summary of the C_T values for the transcripts are provided in Table S2 in the supplemental material. We calculated the fraction of mRNA remaining by comparing the C_T values of our experimental sample (removal of inducer or treatment with actinomycin D) to C_T values of actinomycin D-free control cultures (33) that were treated analogously to the experimental conditions (pelleted, washed, and resuspended in media that contained inducer but not actinomycin D). The fraction of *rpoS* mRNA transcripts (Fig. 2A) and *flaB* mRNA tran-

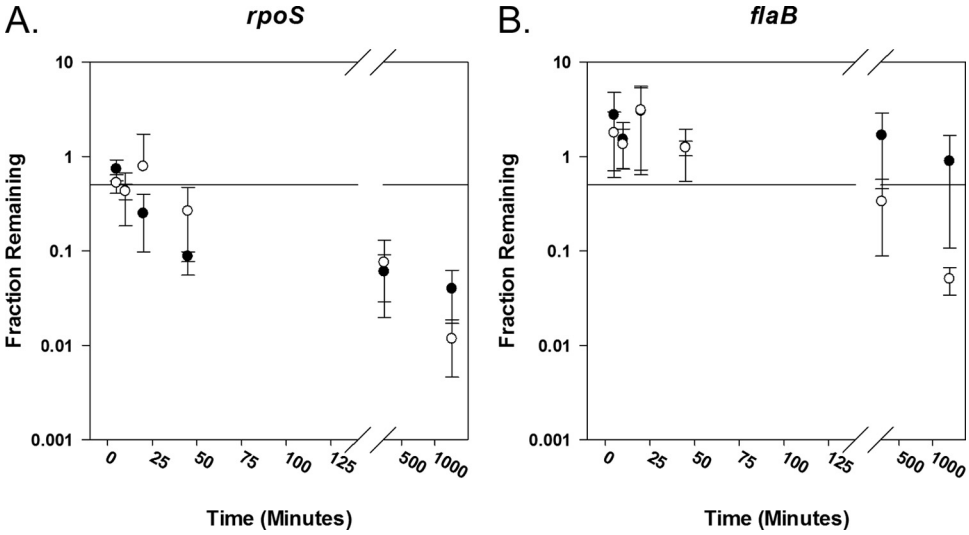

FIG 2 Transcriptional arrest initiated by addition of actinomycin D or by the removal of inducer results in similar patterns of *rpoS* mRNA degradation in strain 297-LK-*flacp-rpoS*. Transcription of *rpoS* was induced by adding 1 mM (final concentration) IPTG to growing 297-LK-*flacp-rpoS* cultures. Twenty-four h later, cells were washed, split, and resuspended in media without inducer or with inducer plus 150 μg ml^{-1} actinomycin D. Aliquots from each culture were collected at time points 5, 10, 20, 45, 240, and 1,260 min after resuspension. The fraction of *rpoS* (A) and *flaB* (B) mRNA remaining at time points after transcriptional arrest brought about by removal of inducer (filled circles) or by addition of actinomycin D (empty circles) was determined. Means ± standard deviations are plotted against time (n = 2; with duplicate qRT-PCRs). A horizontal line representing 50% of the original mRNA level has been plotted as a reference in each graph. Raw data (C_T values) are included in Table S1 in the supplemental material.

scripts (Fig. 2B) remaining following the addition of actinomycin D (open circles) or removal of inducer (filled circles) was plotted against time. Removal of inducer and the addition of actinomycin D both resulted in increases in the C_T values of *rpoS* mRNA with time (see Table S2), indicating that the mRNA levels decreased with time (Fig. 2A). Within minutes, half of the RNA is degraded. In this *rpoS*-inducible strain, *flaB* expression is expected to be independent of the presence of inducer, and we observed that it is (Fig. 2B, filled circles). Following addition of actinomycin D, we observed that the C_T values for *flaB* remained fairly constant for at least 45 min (see Table S2), suggesting that *flaB* mRNA is stable over this period of time (Fig. 2C, open circles). This was consistent with our observation that the amplification curve for *flaB* shifts only slightly to the right and C_T values only modestly increase in strain A3-LS-*flacp-ospC* under actinomycin D treatment (Fig. 1B).

We determined the mRNA decay profiles for transcripts encoding RNA polymerase subunits (*rpoA* and *rpoS*), ribosomal proteins (*rpsD*, *rpsK*, *rpsM*, *rplQ*, and *rpsO*), a nuclease (*pnp*), outer surface lipoproteins (*ospA* and *ospC*), and a flagellar protein (*flaB*) in *B. burgdorferi*. We grew *B. burgdorferi* B31A cells to low cell density ($\sim 5 \times 10^7$ cells ml^{-1}), arrested transcription by treating them with actinomycin D, and purified total RNA at subsequent time points. C_T values for the genes of interest were determined by qRT-PCR using gene-specific primers (see Table S1 in the supplemental material). A complete summary of C_T values is provided in Table S3.

There are qualitative differences in the profiles of mRNA decay for the different genes. To monitor mRNA decay, the fraction of RNA remaining following transcription arrest was calculated by comparing C_T values at given time points to the initial pretreatment value (33) and plotted against time (Fig. 3) in a semilogarithm plot. We observed at least two phases of mRNA decay for each transcript, as indicated by a changing slope in our plots (Fig. 3). Data from the first 45 min were fit as described in the experimental procedures to estimate the rate constant and half-life for each mRNA (Table 2). We inspected the decay curves visually to verify good agreement with the half-lives calculated from the best-fit first-order rate constants.

The *rpoS* and *rpsD* transcripts have an initial rapid decay with a half-life of approximately 1 min (Fig. 3A and D and Table 2). *rpoA*, *rplQ*, *rpsK*, *rpsM*, and *rpsO* mRNA transcripts, the region spanning *rpsO*, and *pnp*, *pnp*, and *ospA* mRNA transcripts show slower degradation over the entire time course (Fig. 3B, C, E to J, and Table 2). These mRNA transcripts decay with half-lives of between 5 and 49 min, which is significantly longer than those of *rpoS* and *rpsD*. Interestingly, *ospC* and *flaB* levels are nearly unchanged for at least 45 min, and no half-lives could be estimated from these curves (Fig. 3K and L). After ~45 min, the transcript levels begin decreasing.

The apparent rapid decay ($t_{1/2} < 10$ min) of the *rpoA*, *rpsD*, *rpsK*, *rpsM*, and *rpoS* transcripts, moderate decay (10 min $< t_{1/2} <$ 50 min) of *rplQ*, *ospA*, *pnp*, and *rpsO* transcripts and the portion of the transcript overlapping *rpsO* and *pnp*, and relative stability of the *ospC* and *flaB* transcripts following actinomycin D addition to cell cultures grown at 35°C suggest that there are gene-specific determinants influencing mRNA decay, as expected. In all cases, the C_T values at 22 h are substantially lower than the values obtained from no-template controls and minus reverse transcriptase controls (see Table S3 in the supplemental material). This suggests that RNA degradation is incomplete for all samples. For cultures grown at room temperature (24°C), even a smaller fraction of the mRNA is degraded following addition of actinomycin D (see Fig. S1).

DISCUSSION

RNA degradation is an important component of gene regulation. Although the importance of gene regulation in the enzootic cycle and in virulence of this bacterium is well recognized, this study is the first to investigate the kinetics of RNA degradation in the spirochete *B. burgdorferi*.

Measurement of mRNA decay requires a method for inhibiting new mRNA synthesis. Previous studies of mRNA decay in bacteria have arrested transcription by employing rifamycin antibiotics (34). Other mRNA decay studies of transcripts controlled by inducible promoters have arrested transcription by removing inducer from cultures to restore transcriptional repression (32, 35). *B. burgdorferi* is naturally rifampin resistant (23), and decay studies employing inducible promoters are limited to genes regulated by an inducible, often artificial, promoter. To circumvent these limitations, we demonstrated that actinomycin D is a suitable transcriptional inhibitor for mRNA decay studies in *B. burgdorferi*. We observed that treatment of *B. burgdorferi* with actinomycin D prior to addition of inducer prevents increases in mRNA levels controlled by an inducible promoter, indicating that it arrests transcription. We also observed similar patterns of decay when we compared the effects of removal of inducer to treatment with actinomycin D on the decay of an inducible transcript.

We measured the mRNA decay patterns for genes encoding RNA polymerase subunits, ribosomal proteins, a nuclease, outer surface lipoproteins, and a flagellar protein (Table 2). One of our most striking results was that transcripts are still detectable 22 h after actinomycin treatment of liquid cultures. Schwartz and coworkers also detected long-lived RNA fragments, suggesting that incomplete digestion of *B. burgdorferi* mRNA is common (16). Our results, together with those previously reported by the Schwartz laboratory, support using caution when using RT-PCR to identify viable *B. burgdorferi*, at least from liquid culture. It is also important to note that in considering mRNA degradation, a single endonucleolytic cleavage within the coding region is sufficient to inactivate an mRNA as a template for translation. This "functional" mRNA half-life is likely to be shorter than the "chemical" half-lives of the fragments we are detecting in our assays. It will be important, in future studies, to compare chemical and functional half-lives of important transcripts.

mRNA decay generally depends upon transcript-specific details (sequences, polyadenylation, secondary structures, ribosome occupancy, RNA binding proteins, etc.) as well as the concentrations, activities, and populations of nucleases in the cell (reviewed in reference 15). Although only a limited set of data has been collected, there is wide variation in the mRNA degradation profiles for the genes we tested. *rpoS*, *rpoA*, *rpsD*, *rpsK*, and *rpsM* mRNAs degrade on the time scale observed for most mRNAs in diverse bacteria (i.e., less than 10 min) (Fig. 3A, B, D, E, and F and Table 2); however, we observed that the degradation of *ospC*, *ospA*, *rplQ*, *rpsO*, *pnp*, and *flaB* fragments is relatively slow (20 min or greater) (Fig. 3C, G, H, I, J, K, and L and Table 2). Slow-growing prokaryotes generally have not shown a corresponding low rate of global mRNA decay (36–39); however, studies of mRNA decay in *M. tuberculosis* show correlations with growth rate and mRNA stability (40). Sherman and coworkers suggest that slower decay

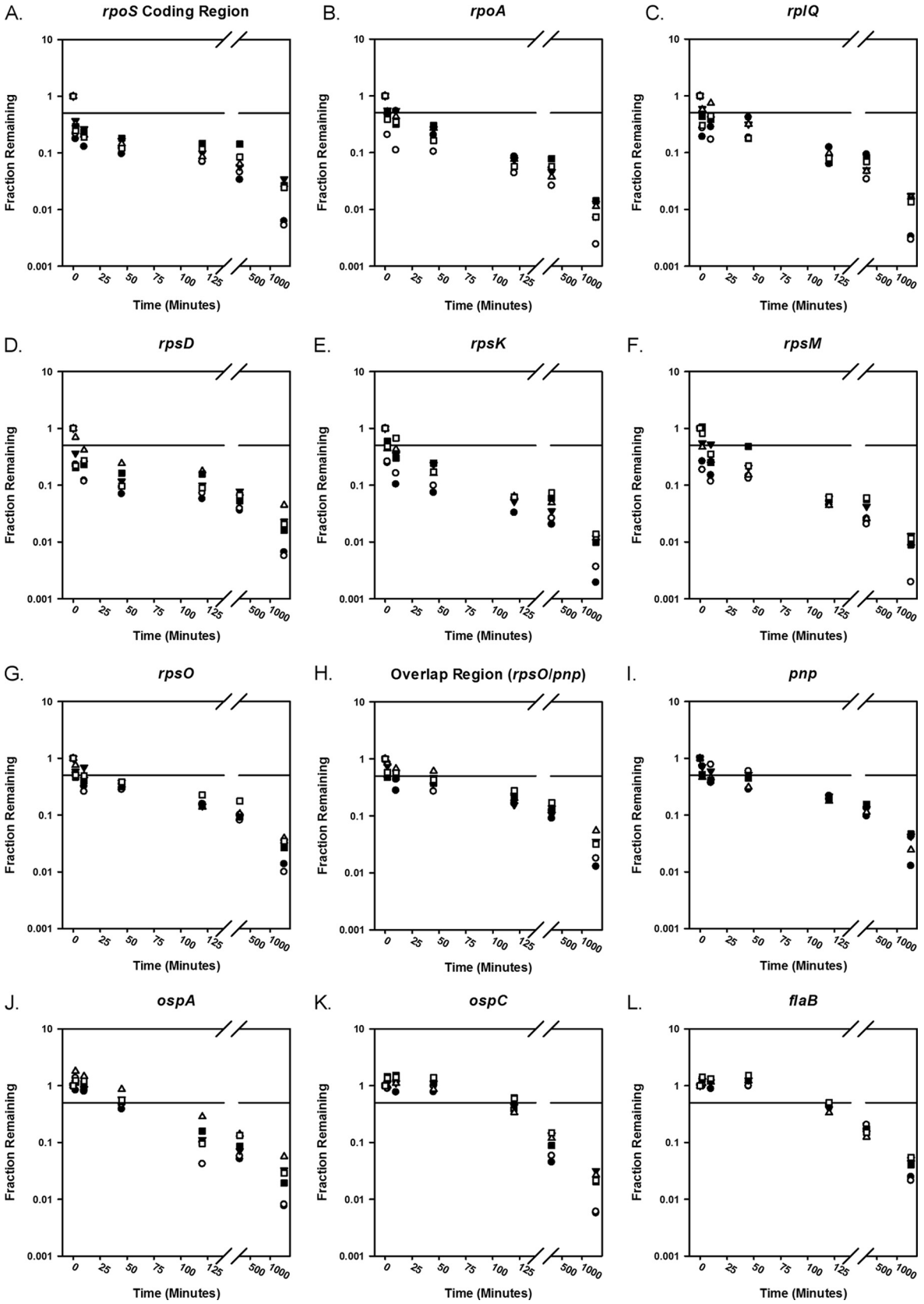

FIG 3 mRNA decay curves for diverse genes in *B. burgdorferi*. The fraction of individual mRNA transcripts remaining at time points following the addition of actinomycin D. Three biological replicates ($n = 3$) were performed (represented by squares, circles, and triangles) with duplicate qRT-PCRs (open versus filled shapes). A horizontal line representing 50% of the original mRNA level has been plotted as a reference. (A) *rpoS* encoding alternative sigma factor RpoS (σ^s). (B) *rpoA* encoding the alpha subunit of RNA polymerase. (C) *rplQ* encoding ribosomal protein L17. (D) *rpsD* encoding ribosomal protein S4. (E) *rpsK* encoding ribosomal protein S11. (F) *rpsM* encoding ribosomal protein S13. (G) *rpsO* encoding ribosomal protein S15. (H) Region overlapping *rpsO* and *pnp*. (I) *pnp* encoding polynucleotide phosphorylase. (J) *ospA* encoding outer surface lipoprotein A. (K) *ospC* encoding outer surface lipoprotein C. (L) *flaB* encoding flagellar protein B.

TABLE 2 Calculated half-lives of mRNA transcripts during the first 45 min of decay

Gene type and name	Predicted first-order rate constant (min^{-1})	Half-life (min)
RNA polymerase subunits		
rpoA	0.087 ± 0.030	7.9
rpoS (−171 UTR)	0.75 ± 0.14	0.92
rpoS coding sequence	0.64 ± 0.12	1.1
Ribosomal proteins		
rplQ	0.034 ± 0.014	20
rpsD	0.52 ± 0.12	1.3
rpsK	0.14 ± 0.043	5.1
rpsM	0.14 ± 0.041	5.0
rpsO	0.025 ± 0.008	27
Overlap		
rpsO and *pnp*	0.019 ± 0.005	38
RNase		
pnp	0.016 ± 0.006	44
Outer surface lipoproteins		
ospA	0.014 ± 0.003	48
ospC	—[a]	—
Flagellar protein		
flaB	—	—

[a] —, not determined.

rates in mycobacteria conserve energy or provide a transcript memory associated with passage of the bacterium from one host to the next (40). Similar strategies may be useful for *B. burgdorferi* during its enzootic cycle.

We observe that the transcript decay is generally not first order; the rates of decay (determined by the slope of the decay curves shown in Fig. 3) all change after the first few hours (decreasing for *rpoA*, *rpsD*, *rpsK*, *rpsM*, *rplQ*, *rpoS*, *pnp*, and *rpsO*, the region overlapping *rpsO*, and *pnp* and *ospA*; increasing for *flaB* and *ospC*). For *flaB* and *ospC*, the increases in the degradation rates with time may simply result from differences in the affinities of nucleases for different transcripts. At early times, transcripts that are good nuclease substrates will be bound and processed. As these good substrates are used up, the effective nuclease concentration will increase to facilitate degradation of poorer substrates. The observed decreases in the rates of degradation of some of the transcripts at long times following transcriptional arrest may result from cellular global changes (e.g., the concentrations or activity of nucleases) or transcript-specific effects (e.g., ribosome occupancy). The significant differences in the decay profiles suggest that these transcripts will be useful for determining the transcript-specific details (e.g., nuclease recognition sequences and roles of secondary structure) that influence rates of mRNA decay in more detailed mechanistic studies.

mRNA lifetimes appear to be correlated with the function of the protein that they encode in *E. coli* (36), *Lactococcus lactis* (41), *Prochlorococcus* (39), and *Bacillus cereus* (42), although this correlation was not observed in *B. subtilis* (37). Generally, we observed rapid decay of transcripts encoding ribosomal proteins and RNA polymerase subunits in *B. burgdorferi*; however, *rplQ* and *rpsO* mRNA degradation was much slower than that observed for mRNAs encoding other ribosomal proteins (Table 2).

The enzootic cycle involves well-characterized changes in expression of *rpoS*, *ospC*, and *ospA*, and we investigated the decay of these transcripts to determine whether transcripts associated with gene expression in specific hosts have similar mRNA decay profiles. As ticks take on a blood meal, RpoS in *B. burgdorferi* cells increases (5) and influences the mRNA levels of virulence genes (43). *ospC* mRNA levels increase following feeding (5) and remain high during early mammalian infection. The precise function of OspC is not known, but it is required for mammalian infection (44). OspA is essential for both acquisition of *B. burgdorferi* by the tick and the spirochete's survival in the tick; OspA interacts with a tick midgut protein (45–47). OspA is synthesized by *B. burgdorferi* within unfed ticks and in culture at lower temperatures (e.g., 23°C), and OspA levels decrease upon temperature upshift in culture only if RpoS is expressed (7). Our data indicate that the mRNA decay profiles at 35°C of the genes essential for mammalian infection are quite different from each other (*rpoS* $t_{1/2}$, ~1 min; *ospC* $t_{1/2}$, >50 min). Decay of the tick-specific *ospA* at 35°C ($t_{1/2}$, 48 min) is more similar to that of *ospC* than that of *rpoS*, indicating that mRNA decay rates are independent of the gene's function in the enzootic cycle at one set of growth conditions. These longer-lived transcripts encoding outer surface lipoproteins may be important in determining the time scale for *B. burgdorferi* to transition between tick and vertebrate hosts. For example, the longer-lived transcripts may delay the reduction in OspA levels associated with initiation of *B. burgdorferi* migration from the tick midgut to salivary glands.

Correlations between RNA decay and gene function may result from operon structure rather than specific gene function (as proposed for *B. subtilis* [37]). Because the 5′ and 3′ ends of most genes in *B. burgdorferi* have not been mapped, the operon configurations were predicted (Fig. 4) using the Prokaryotic Operon Database (48) and Microbes Online Operon Predictions (49) and modified by 5′ end mapping data for *rpoS* (50) and *rpsM* (data not shown). We observed variability in the time scale of decay of genes that are expected to be found on a polycistronic transcript. Specifically, decay at the 5′ ends of transcripts is faster than at the 3′ ends (Fig. 4); e.g., compare 5′ *rpsM* ($t_{1/2}$ of 5 min) to 3′ *rplQ* ($t_{1/2}$ of 20 min). Similar observations have been reported previously for *E. coli* (36) and *Prochlorococcus* (39). Our limited data set suggests that there is a relationship between decay rates and operon configuration, but we are less confident that there is a correlation with decay rates and gene function. A wider data set evaluating decay of the entire transcriptome, especially under diverse conditions, will be required to determine whether there are more generally observed correlations with gene function or operon configuration.

B. burgdorferi contains the orthologs to endoribonucleases RNase III and RNase Y but is missing an ortholog to RNase E (Table 1) (18), suggesting that its mechanisms of mRNA degradation are more similar to those observed in *B. subtilis* than those in *E. coli*. However, *B. burgdorferi* is missing close orthologs to the *B. subtilis* ribonucleases J1 and J2. Analysis of the roles of most ribonucleases (e.g., J1 and J2 in *B. subtilis* and RNase III and RNase Z in *E. coli*) indicates that they contribute to mRNA degradation pathways, at least for some genes (51–53). We propose that deletion or conditional expression of *B. burgdorferi* nucleases (Table 1) will help us determine which nucleases are most important in degrading these transcripts.

Although global changes in mRNA lifetime may occur as a result of environmental shifts, individual mRNA lifetimes in other

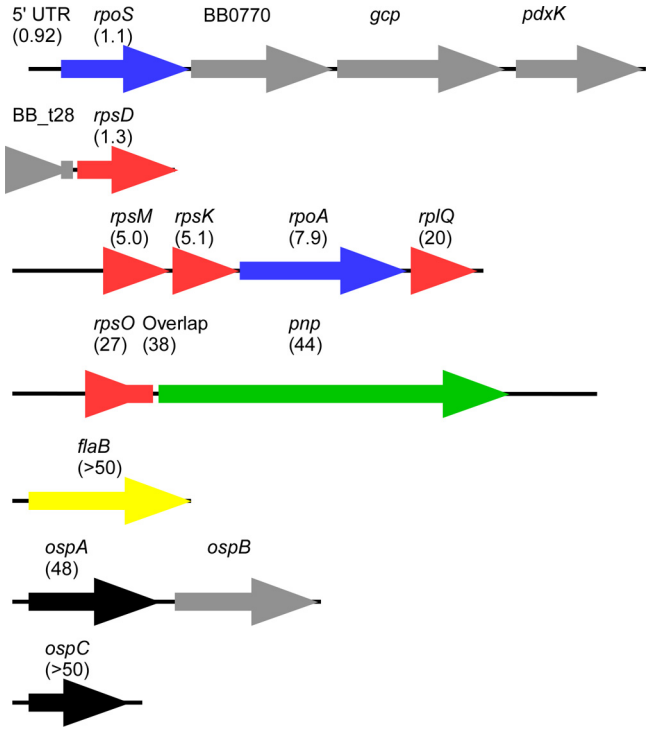

FIG 4 Comparison of mRNA half-lives in polycistronic transcripts. The expected operon configuration for genes used in this study are shown with the calculated half-life of the amplified product labeled in parentheses. Genes encoding RNA polymerase subunits are blue, ribosomal proteins are red, nucleases are green, outer surface lipoproteins are black, and flagellar proteins are yellow. Gene loci that were not tested are colored gray.

bacteria have been identified from microarrays to be particularly influenced by specific environmental conditions, indicating that adaptation is mediated by mRNA decay (41, 54–56). We measured mRNA decay from cultures grown at room temperature. At room temperature, actinomycin D prevented induction of *ospC* in strain A3-LS-*flacp-ospC*, indicating that it serves to arrest transcription at this temperature (data not shown). *B. burgdorferi* cells grown at room temperature retained motility for more than 20 h following treatment with actinomycin D and showed some evidence of continued cell division (data not shown). We observed that the rate and extent of mRNA degradation of *rpoS*, *ospC*, *ospA*, *flaB*, *pnp*, and *rpsO* was much lower for cultures grown at room temperature (see Fig. S1 in the supplemental material), suggesting that the effects of temperature on mRNA degradation in *B. burgdorferi* are significant in the enzootic cycle.

Bacteria rapidly respond to changes in environmental conditions using a combination of rapid degradation of mRNA and transcriptional control to modulate gene expression. This is observed even in slow-growing bacteria and appears to be independent of doubling time. (reviewed in reference 15). Gene regulation studies in *B. burgdorferi* have identified transcriptional regulation mediated by the alternative sigma factors RpoS and, to a lesser extent, RpoN, as well as a DNA binding protein (BosR) (reviewed in reference 3). Although *B. burgdorferi* has evolved with a limited subset of ribonucleases, DNA binding domains, or orthologs to transcriptional regulators found in other bacteria (3, 19), the mRNA levels of hundreds of genes are altered by changing environmental conditions. We expect that further understanding of the roles of mRNA degradation on gene expression and regulation during the enzootic cycle will be important in understanding the basic biology of this important pathogen.

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