Translation inhibitors stabilize Escherichia coli mRNAs independently of ribosome protection
(mRNA stability/ RNase E/poly nucleotide phosphorylase/ autoregulation)

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ABSTRACT  Translation inhibitors such as chloramphenicol in prokaryotes or cycloheximide in eukaryotes stabilize many or most cellular mRNAs. In Escherichia coli, this stabilization is ascribed generally to the shielding of mRNAs by stalled ribosomes. To evaluate this interpretation, we examine here how inhibitors affect the stabilities of two untranslated RNAs, i.e., an engineered lacZ mRNA lacking a ribosome-binding site, and a small regulatory RNA, RNAI. Whether they block elongation or initiation, all translation inhibitors tested stabilized these RNAs, indicating that stabilization does not necessarily reflect changes in packing or activity of translating ribosomes. Moreover, both the initial RNase E-dependent cleavage of RNAI and lacZ mRNA and the subsequent attack of RNAI by polynucleotide phosphorylase and poly(A)-polymerase were slowed. Among various possible mechanisms for this stabilization, we discuss in particular a passive model. When translation is blocked, rRNA synthesis is known to increase severalfold and rRNA becomes unstable. Meanwhile, the pools of RNase E and polynucleotide phosphorylase, which, in growing cells, are limited because these RNases autoregulate their own synthesis, cannot expand. The processing/degradation of newly synthesized rRNA would then titrate these RNases, causing bulk mRNA stabilization.

Drugs that block peptide bond synthesis, such as cycloheximide in eukaryotes or chloramphenicol in prokaryotes, are known to cause stabilization of many (and perhaps all) cellular mRNAs (1, 2). This effect can be mimicked in the absence of drugs by mutations in the translational apparatus that slows or blocks ribosome movement (3, 4). However, the mechanisms underlying this stabilization remain unknown. In yeast and higher cells, mRNAs can be stabilized not only by cycloheximide, which causes ribosome stalling, but also by puromycin, which strips mRNAs from ribosomes (2). On this basis, it has been proposed that drugs stabilize mRNAs not by altering the packing or activity of the ribosomes translating them, but by somehow inhibiting the mRNA degradation machinery itself (“trans” effect) (2).

In contrast, in prokaryotes, it is generally assumed that translation inhibitors affect mRNA stability mainly, if not entirely, by altering the packing or activity of translating ribosomes (“cis” effect). This view originates from two lines of evidence. First, drugs now affect mRNA stability differently depending on how they inhibit translation. Thus, whereas chloramphenicol, fusidic acid, and tetracycline, which stall ribosomes on mRNAs, yield stabilization, puromycin and kasugamycin, which strip mRNAs of ribosomes, have the opposite effect (5, 6). Second, ribosomes can be stalled or pulled off even in the absence of inhibitors, and in several cases these changes affect mRNA stability like the corresponding classes of inhibitors. Thus, it has been reported that, in Bacillus subtilis, ribosome stalling can stabilize downstream mRNA regions (7, 8). Conversely, in Escherichia coli, mutations in the ribosome-binding site, which reduce initiation site frequency, also generally lower mRNA stability (see ref. 1 for discussion).

Although the above data argue in favor of a cis mechanism, they do not exclude the existence of superimposed trans effects. Such effects should be revealed most clearly by testing the effect of inhibitors upon the stability of untranslated mRNAs: were cis effects exclusive, this stability should be insensitive to translation inhibitors, whereas if trans effects existed, it would remain sensitive. A practical difficulty in this approach is that the E. coli RNA polymerase often is ineffective in synthesizing long stretches of untranslated mRNA, because of transcription–translation coupling (transcriptional polarity; ref. 9). Previously, we advocated the use of T7 RNA polymerase (T7 RNAP), which is insensitive to polarity, to bypass this difficulty (10). Here we use this strategy to investigate the effect of translation inhibitors upon the stability of a lacZ mRNA lacking a ribosome-binding site so that translation cannot occur. We further substantiate our results by extending our study to RNAI, a small untranslated RNA, the decay of which is well characterized (11–15).

MATERIALS AND METHODS

Plasmids and Strains. Plasmid pGM102 (16) was a generous gift of G. Mackie (Univ. of British Columbia, Vancouver, Canada). Strain ENS32 (BL21(DE3) lacZ::Tn10, malPpΔ534::Pγ-lacZΔ) is described elsewhere (17, 18). To create strain ENS304, we first replaced the lacZ-lacY-lRNAI-γg4 region of plasmid pTRNAI by a fragment containing the RNAI gene. This fragment was obtained after amplification of the relevant region of plasmid pBR322, by using 5′-CGCTGATACAGTATGGTGTACCGCGG-3′ and 5′-CGCTGATACAGTATGGTGTACCGCGG-3′ as upstream and downstream primers, respectively. This fragment was digested by BamHI and SnaBI and inserted within the same sites of pTlacZ-Arg5, yielding pTTRNAI. Using published procedures (10), the pTRNAI fragment from upstream of the Pγ promoter to downstream of the terminators then was transferred onto the chromosome of MO20 (19), a Lac− Tet− derivative of strain BL21(DE3) (20). This transfer yielded ENS304 (BL21(DE3) ΔlacZ::malPpΔ534::Pγ-lRNAI). The chromosome structure of the final strains is schematized in Fig. 1. To obtain the nce-1 derivative of ENS304, the same con-
struct as above was transferred onto the chromosome of MO20–1, the rne-1 derivative of MO20 (21). The pcnB-1 derivative of ENS304 was obtained by transducing the pcnB80 mutation (22).

**Cell Growth.** Cells were grown at 37°C in a synthetic-rich medium (23). The generation time in the absence of translation inhibitors was ca. 40 min whether isopropyl β-D-thiogalactoside (IPTG) was present or not. Translation inhibitors were used at the following concentrations: chloramphenicol, 170 μg/ml; kasugamycin, 10 mg/ml; tetracyclin, 50 μg/ml. The rne-1 cells was grown either continuously at 37°C (semipermissive conditions) or at 30°C followed by a 30-min shift to 42°C before harvest (nonpermissive conditions), as indicated.

**RNA Isolation and Analysis.** For RNA analysis, IPTG (1 mM) was added to exponentially growing cells (OD₆₀₀ ca. 0.4), 15 min before drug addition, except otherwise stated; this time is long enough for transcripts from lacZΔ (strain ENS32) or RNAI (strain ENS304) to genes reach the steady-state level. After drug addition, total RNA was extracted at timed intervals from aliquots of the cultures, electrophoresed on 1.8-kb nylon membrane (23, 24). For probing the lacZΔ RNA, the internal 1.8-kb HindII fragment of the lacZ gene was 32p-labeled with the BRL multiprime labeling kit. RNAI was probed with oligonucleotide 5'-32PGATCAAGAGGTAC-CAACTC-3' (Fig. 3a). Hybridization signals were quantified with a BAS 1000 Imager (Fuji). For primer extension analysis, RNA (5 μg) from either ENS304 cells or from ENS32 cells harboring plasmid pBR322 was hybridized with an excess of the above RNAI probe. Hybrids were extended and analyzed as in ref. 25.

To follow the decay of the lacZΔ or RNAI transcripts in the presence of inhibitors, cells that had been incubated in inhibitor-containing medium were rapidly filtrated through 0.45-μm HA type circular filters (Millipore) and resuspended into the same prewarmed medium lacking IPTG (19). This transfer, which switches off of the T7 promoter, takes typically 30 s. At timed intervals, total RNA was then extracted and analyzed as above. The same protocol was used with control cultures without inhibitors.

**RESULTS**

**An Untranslated lacZ mRNA Is Stabilized by Translation Inhibitors.** Elsewhere we describe the construction of an E. coli strain (ENS32) in which the malA chromosomal region harbors an untranslated version of the lacZ gene (and part of the lacY gene) fused downstream of a T7 late promoter (P₇7) (17, 18). ENS32 is a derivative of the T7 RNAP-producing strain BL21(DE3) (20), in which the genuine lacZ gene has been inactivated. The structure of the P₇7-lacZ construct in ENS32 is described in Fig. 1; the following two points are noteworthy. First, the P₇7-lacZ junction is so engineered that the high-affinity lac repressor-binding site is centered in close proximity to P₇7 (i.e., at the 13th transcribed nucleotide). This location allows P₇7 to be switched on or off by adding or removing the lac operon inducer IPTG, without resorting to the use of general transcription inhibitors (26). Second, a 35-nt-long region encompassing the Shine–Dalgarno sequence and initiator codon has been deleted: as a result, no β-galactosidase activity is observed after IPTG induction, nor can β-galactosidase-related polypeptides be detected on Western blots (not shown). Yet, this truncated lacZ gene, hereafter referred to as lacZΔ, is transcribed efficiently because T7 RNAP is insensitive to polarity (10).

Total RNA was extracted from ENS32 cells growing in the presence of IPTG and analyzed on Northern blots. The lacZΔ mRNA appears as a faint smear, with very little material matching the size expected for the full-length transcript (4.2 kb) (Fig. 2a, lane 0). Elsewhere we noted that the smear corresponds mainly to nascent mRNA molecules. The absence of full-length transcripts reflects the extreme sensitivity of these nascent chains to RNase E attack (ref. 18; see also Fig. 6, rightmost lane); few of them survive undegraded for the time required for completing transcription (i.e., less than 20 s at 37°C when T7 RNAP is used; ref. 27). Lethal concentrations of chloramphenicol, tetracycline, or kasugamycin then were added, and aliquots of the culture subsequently were removed at timed intervals for Northern blot analysis (Fig. 2a; tetracycline experiment is not illustrated). After the addition of any of the drugs, the lacZΔ mRNA pattern gradually changed with time, with higher-molecular-weight species being progressively favored over smaller ones. In particular, the abundance of the full-length transcript (4.2 kb) increased considerably compared with that of lower-molecular-weight species (e.g., ~1 kb). In addition, a 3.2-kb species originating from a 3' processing of the full-length transcript and encompassing the lacZ mRNA proper (28) accumulated gradually. We regard these changes as indicating that a higher proportion of transcripts reach full-length size before starting to decay, i.e., that the message is stabilized.

To ascertain this stabilization, we compared directly the decay of the lacZΔ mRNA in ENS32 cells in the absence of drug or after a 20-min kasugamycin treatment. To this end, we switched off rapidly the P₇7 promoter by transferring cells from IPTG-containing to IPTG-free medium. At timed intervals after this transfer, aliquots of the cultures were processed as above, and the decay of the lacZΔ mRNA was visualized on Northern blots (Fig. 2b). In control cells, the faint smear corresponding to the lacZΔ mRNA was no longer visible 1 min after IPTG removal, illustrating the instability of this transcript. In contrast, in kasugamycin-treated cells, the transcript appears far more stable: half-lives of 2 and 5 min can be estimated for the 4.2- and 3.2-kb species, respectively.

Thus, in spite of the fact that the lacZΔ mRNA is not translated, it can be stabilized by translation inhibitors. Moreover, all inhibitors are equivalent in this respect, whether they
block initiation or elongation. We conclude that translation inhibitors presumably stabilize this mRNA via trans effects.

Construction and Properties of a Chromosome-Borne RNAI Gene. Because the rate-limiting step in the *lac*Δ mRNA decay is controlled by RNase E (18), the above experiment suggests that drugs somehow block the activity of this enzyme. However, the RNase E cleavage sites on this mRNA have not been characterized, nor is it known how the resulting products are subsequently degraded. To get a deeper insight into the way translation inhibitors stabilize mRNAs, we examined how they affect the degradation of yet another untranslated RNA, RNAI. This 108-nt-long RNA, which down-regulates the copy number of ColEl-derived plasmids, is metabolically unstable, like an mRNA. For consistency, the same genetic system used for the *lac*Δ mRNA also was used here. We constructed a strain (ENS304) similar to ENS323 except that the *lac*Δ gene is replaced by the RNAI gene from the first nucleotide to the rho-independent terminator (Figs. 1 and 3a). In addition, the *lac* operator is still present between the Pr7 promoter and the RNAI gene, so that the transcript carries 31 nt at its 5′ end that are not present in the genuine pBR322 RNAI (Fig. 3a). This increase in length can be visualized by primer extension (Fig. 4) by using a probe complementary to the middle of RNAI (Fig. 3a). At their 3′ end, the two transcripts are identical because T7 RNAP recognizes the RNAI terminator (29).

The decay pathway of the genuine RNAI (half-life ca. 2 min at 37°C) is well characterized. First, an RNase E-dependent cleavage near the 5′ end generates a 103-nt species (RNAI–31) from ENS304 cells. The three hairpins are tentatively drawn based on the structure of the genuine RNAI. The positions of the major and minor RNase E cleavage sites, generating RNAI–5 and RNAI–34, respectively, are indicated by an arrow. The 5′ extension corresponds mostly to the sequence transcribed from the *lac* operator; the genuine RNAI sequence starts 5 nt upstream of the major RNase E site. The sequence complementary to the oligonucleotide probe used for Northern blots and primer extensions is overlined. (**Materials and Methods** for details). At the indicated time after drug addition, the *lac*Δ mRNA was visualized on Northern blots, using the same oligonucleotide as a probe. (Left) or in cells that have been treated with kasugamycin for 20 min (Right). At zero time, transcription has been switched off by transferring the cells to an identical medium lacking IPTG. All symbols are as in a.

Fig. 2. (a) Effect of addition of chloramphenicol (Left) or kasugamycin (Right) upon accumulation of the *lac*Δ mRNA (see **Materials and Methods** for details). At the indicated time after drug addition, the *lac*Δ mRNA was visualized in total RNA from ENS32 cells by using Northern blots. The position of the full-length operon transcript (4.2 kb) and of a processed species corresponding to the *lac* mRNA proper (3.2 kb), as well as that of the 23S and 16S RNAs, are indicated. Controls showed that M00, the parent strain lacking the *P*7–*lacZ* construct, produced no *lac*-hybridizing signal under these conditions (cf. 21). (b) Northern blot showing the decay of the *lac*Δ mRNA in control cells (Left) or in cells that have been treated with kasugamycin for 20 min (Right). At zero time, transcription has been switched off by transferring the cells to an identical medium lacking IPTG. All symbols are as in a.

Fig. 3. (a) Schematic structure of the engineered RNAI (RNAI–31) from ENS304 cells. The three hairpins are tentatively drawn based on the structure of the genuine RNAI. The positions of the major and minor RNase E cleavage sites, generating RNAI–5 and RNAI–34, respectively, are indicated by an arrow. The 5′ extension corresponds mostly to the sequence transcribed from the *lac* operator; the genuine RNAI sequence starts 5 nt upstream of the major RNase E site. The sequence complementary to the oligonucleotide probe used for Northern blots and primer extensions is overlined. (b) Northern blots showing the RNAI–31, RNAI–5, and RNAI–34 species (arrow) in the wild-type ENS304 strain and *penB*80 derivative (Left) or in the wild-type and *rne*–1 derivative (Right). Cells were grown steadily at 37°C (Right) Cells were grown at 30°C and then shifted to 42°C 30 min before harvest. Signal intensities in both are comparable directly. The symbols (+) and (−) denote the presence or absence of IPTG in the growth medium. In the bottom panels, the same membrane has been reprobed with a 5S rRNA-specific probe.
detected in the pcnB strain (Figs. 3b and 4). All these features match those reported for the genuine RNAI, indicating identical decay mechanism.

Translation Inhibitors Stabilize RNAI. Translation inhibitors were then added to exponentially growing cultures of ENS304 cells, and the concentrations of RNAI_{131} and RNAI_{-5} were recorded as a function of time by using Northern blots. Again, inhibitors of translation initiation (kasugamycin) or elongation (tetracycline, chloramphenicol, fusidic acid) gave similar results: only experiments using chloramphenicol and kasugamycin are illustrated (Fig. 5a). In the presence of drug, both RNAI_{131} and RNAI_{-5} accumulated with time. Accumulation was most pronounced for RNAI_{-5}, which after 30 min was almost as abundant as RNAI_{131}. The accumulation of RNAI_{131} and RNAI_{-5} in inhibitor-treated cells is reminiscent of that of the lacZΔ mRNA (Fig. 2a). To check that it similarly reflects a stabilization, transcription was switched off after 20 min of chloramphenicol treatment by removing IPTG, and the subsequent decay of RNAI_{131} and RNAI_{-5} was examined. The corresponding half-lives (ca. 12 and 8 min, respectively) were much longer than in cells that had not been treated with chloramphenicol (2 and <1 min, respectively) (Fig. 5b).

Similar results were obtained after blocking T7 RNAP transcription with actinomycin D, a general transcription inhibitor (not shown). We conclude that both the RNase E-mediated cleavage of RNAI_{131} and the subsequent exonucleolytic degradation of RNAI_{-5} are slowed by translation inhibitors, with the latter process being affected most. Incidentally, when the same Northern blots were reprobed with a 5S rRNA-specific probe for standardization purposes (cf. 19), we found that the proportion of 5S rRNA in total RNA (i.e., essentially rRNA) rises steadily after drug addition. The increase was as much as 2-fold after 20 min, i.e., half the generation time in the absence of drug (Fig. 5a). This observation is consistent with former work showing that rRNA transcription is boosted vigorously after drug addition, but that large rRNAs (i.e., 16S and 23S rRNAs) are then unstable and do not accumulate (32).

As a control, the effect of drugs upon accumulation of genuine RNAI from pBR322 was examined. Both RNAI and RNAI_{-5} could be visualized in pBR322-harboring cells by primer extension (Fig. 4). Kasugamycin treatment caused a preferential accumulation of RNAI_{-5}, indicating that this species, like its T7 RNAP-synthesized counterpart, is stabilized with respect to its precursor.

Effect of RNase E Overexpression. Because the decay of the lacZΔ mRNA and RNAI_{131} is controlled by RNase E, the stabilization of these species in drug-treated cells might reflect a shortage in RNase E activity under these conditions. To evaluate this point, we repeated the experiment shown in Fig. 2a with cells overproducing RNase E. RNase E normally autoregulates its own synthesis, but this regulatory loop can be
bypassed by using plasmid pGM102, which bears the rne gene under the control of the P T7 promoter (16). After a 30-min induction of the plasmid-borne rne gene in ENS32 cells, RNase E was overproduced substantially (Fig. 6). When these cells then were treated with kasugamycin, the accumulation of the 4.2- and 3.2-kb species was less than in cells harboring a control plasmid lacking the rne gene (Fig. 6). Measurement of mRNA decay under these circumstances revealed that this lesser abundance reflects a lower stabilization (not illustrated). Therefore, the stabilization of the 4.2- and 3.2-kb species in drug-treated cells presumably reflects a limited availability of RNase E activity. Interestingly, drug treatment of RNase E-overproducing cells also yielded a marked accumulation of the 4.2- and 3.2-kb species, reflecting loss of autocatalysis.

**FIG. 6.** (Left) Northern blot showing the accumulation of lacZ mRNA in ENS32 cells harboring either the RNase E-overexpressing plasmid (pGM102) or the parent plasmid pET11a (control). Transcription from the plasmid (and from the lacZΔ gene) was induced 30 min before kasugamycin addition. The RNA was analyzed just before (‘0’) or 60 min after (‘60’) this addition. RNA from the rne-1 derivative of ENS32 (18) harboring pET11a and grown in the absence of kasugamycin at the semi-permissive temperature of 37°C also is shown as a control (fifth lane). Note the stabilization of the lacZΔ mRNA in this strain as compared with the rne-1 strain (first lane). (Right) Western blot showing the accumulation of RNase E in the same samples used in Left (0 time). Note the accumulation of RNase E in the rne-1 strain, reflecting loss of autocatalysis.

**Results**

Why Is RNA Stabilized After a Translation Block? The stabilization of the lacZΔ mRNA in drug-treated cells reflects a shortage in RNase E activity under these conditions (Fig. 6). Similarly, the RNA1−31 and RNA1−5 species presumably are stabilized because of a shortage in RNase E and PNPase [or poly(A) polymerase] activities, respectively. We discuss below two plausible, nonexclusive interpretations for these shortages.

*Translational block may inhibit the degradation machinery.* Assuming that a translation block inhibits the degradation machinery, then the observation that both the endonucleolytic, RNase E-dependent cleavage of RNA1−31 or lacZΔ mRNA and the exonucleolytic, PNPase−poly(A) polymerase-dependent degradation of RNA1−5 are affected suggests that the target of this inhibition may be the “degradosome,” a multienzymatic complex associating RNase E and PNPase together with other proteins (34–36). Why would the degradosome be inhibited under these circumstances? Conceivably, it may contain a functionally unstable component and therefore require ongoing protein synthesis for full activity. A similar interpretation often has been offered for rationalizing drug-mediated mRNA stabilization in eukaryotes (2). Alternatively, the presence of drugs may induce allosteric changes in the degradosome that reduce its activity. Interestingly in this respect, drug treatment causes an expansion of the ATP (and GTP) pool (37); this expansion may affect the activity of several degradosome proteins that use ATP as substrate or cofactor (35, 38, 39). Finally, it is possible that drugs somehow hamper degradosome access to mRNAs. Indeed, biochemical work indicates that mRNAs can be sequestered in RNase-resistant complexes, the abundance of which vary with the physiological state of the cell (40).

*A block in translation may saturate the degradation machinery.* Four years ago, D. Court pointed out that, because RNase III controls the decay of its own mRNA, it should never be present in excess in the cell; rather, its pool should adjust tightly to that of its main substrate, tRNA (41). Similarly, RNase E autoregulates its own synthesis by controlling the decay of its mRNA (42). It is therefore plausible that its concentration in growing cells continuously adjusts to that of its substrates. One of these substrates is again tRNA, because RNase E is responsible for the maturation of 5S rRNA from its immediate precursor, 9S rRNA. Now, when protein synthesis is blocked, inhibit translation. Thus, ribosome-stalling drugs (e.g., chloramphenicol, tetracycline, or fusidic acid) stabilize these mRNAs individually or in bulk, whereas drugs that strip mRNAs of ribosomes (e.g., kasugamycin or puromycin) tend to destabilize them (5, 6, 33). On this basis, it has been assumed that drugs affect the stability of mRNAs by altering the packing or activity of the ribosomes that translate them (cis mechanism). Our results are not at odds with this view; simply, with translated mRNAs, cis and trans effects presumably superimpose to each other. In the case of ribosome-stalling drugs, they would cooperate in stabilizing mRNAs, whereas in the case of mRNA-stripping drugs they would antagonize each other. Consistently with this latter view, we note that the reported half-life of the translated lacZ mRNA immediately after kasugamycin addition [ca. 30 s (5, 33)], though shorter than that of the normally translated lacZ mRNA (1.2 min), is presumably much longer than the half-life of a lacZ mRNA that would simply be stripped of its ribosomes (in the absence of drugs the half-life of the lacZΔ mRNA is ≤20 s; see Results). Incidentally, in these former studies, changes in lacZ mRNA stability could be recorded only immediately after kasugamycin addition, because of polarity effects. In contrast, our experimental setting allows long-term effects of drugs to be analyzed. After 20 min of kasugamycin treatment, the half-life of the lacZΔ mRNA (ca. 5 min) is longer than that of the normally translated lacZ mRNA in the absence of drug. This observation suggests that trans effects overcome cis effects upon standing, resulting in net stabilization.

**DISCUSSION**

Drugs That Block Protein Synthesis Stabilize Untranslated mRNAs. We have shown here that three untranslated RNAs, i.e., the lacZΔ mRNA and the RNA1−31 and RNA1−5 species, are stabilized in E. coli by inhibitors of protein synthesis. Similar effects were observed with all drugs tested, irrespective of how they inhibit translation. From these data, it is clear that drugs can stabilize mRNAs by acting directly on mRNA degradation machinery (trans effect). Remarkably, both an endonucleolytic process (the RNase E-mediated cleavage of lacZΔ mRNA and RNA1−31) and an exonucleolytic process (the PNPase-poly(A) polymerase-mediated degradation of RNA1−5) are slowed by the drugs. Moreover, RNA1−5 was stabilized to a greater extent than RNA1−31, suggesting a preferential inhibition of the exonucleolytic process in this case.

It has been reported previously that drugs affect the stability of translated mRNAs differently, depending on how they

the concentration of RNase E can no longer adjust to that of its substrates. Meanwhile the level of RNA synthesis increases severalfold because of a boost in rRNA synthesis; moreover, the newly synthesized rRNA is then unstable, presumably because it cannot assemble with ribosomal proteins (ref. 32; see Results). Plausibly, this increase in rRNA synthesis reflects the expansion of the GTP and ATP pools, which have been shown recently to control the activity of the rnr P1 promoters (43). We then hypothesize that the higher synthesis of rRNA in drug-treated cells will increase the demand for the processing activity of RNase E, precisely under circumstances in which its pool cannot expand; saturation of this activity will ensue. rRNA is probably a far better substrate for RNase E than bulk mRNA (the 9S rRNA species is difficult to detect on Northern blots; from the intensity of the corresponding signal vs. that of the 5S rRNA, we estimate that in growing cells its lifespan cannot exceed a few seconds, vs. minutes for bulk mRNA). Therefore, saturation of RNase E activity primarily will impair mRNA decay. A similar reasoning may explain why the PNPase–poly(A) polymerase-dependent degradation of RNAI-s is slowed in the presence of drugs. Like RNase E and RNase III, PNPase is known to autoregulate its own synthesis (44), and in drug-treated cells it may become saturated with unstable rRNA, resulting in mRNA stabilization.

Note Added in Proof. While this work was in press, Bessarab et al. (47) reported that the E. coli degradosome contains rRNA fragments, suggesting that it participates in rRNA degradation. The unstable rRNA synthesized after a translational block might then titrate the degradosome itself, explaining why both RNase E- and PNPase-mediated pathways of mRNA decay become inhibited under these conditions.

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