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Biochemistry

Translation inhibitors stabilize *Escherichia coli* mRNAs independently of ribosome protection

(mRNA stability/RNase E/polynucleotide 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 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*::Tn*10*, *malP*pΔ*534*::P_{T7}*lacZ*Δ) is described elsewhere (17, 18). To create strain ENS304, we first replaced the *lacZ-lacY′-tRNA*^{Arg5} region of plasmid pTLacZ-Arg5 (19) by a fragment containing the RNAI gene. This fragment was obtained after amplification of the relevant region of plasmid pBR322, by using 5′-CGCGGATCCACAGTATTTGGTATCTGCG-3′ and 5′-CGCTACGTAACAAAAAAACCACCGCT-3′ as upstream and downstream primers, respectively. This fragment was digested by *Bam*HI and *Sna*BI and inserted within the same sites of pTLacZ-Arg5, yielding pTRNAI. Using published procedures (10), the pTRNAI fragment from upstream of the P_{T7} promoter to downstream of the terminators then was transferred onto the chromosome of MO20 (19), a Lac$^-$ Tet^s derivative of strain BL21(DE3) (20). This transfer yielded ENS304 (BL21(DE3) Δ*lacZ*:*malP*pΔ*534*::P_{T7}*RNAI*). The chromosome structure of the final strains is schematized in Fig. 1. To obtain the *rne-1* derivative of ENS304, the same con-

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Abbreviations: IPTG, isopropyl β-D-thiogalactoside; T7 RNAP, T7 RNA polymerase; PNPase, polynucleotide phosphorylase.

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struct as above was transferred onto the chromosome of MO20–1, the *rne-1* derivative of MO20 (21). The *pcnB*$^-$ 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 (OD_{600} *ca.* 0.4), 15 min before drug addition, except otherwise stated; this time is long enough for transcripts from *lacZ*Δ (strain ENS32) or RNAI (strain ENS304) genes to reach the steady-state level. After drug addition, total RNA was extracted at timed intervals from aliquots of the cultures, electrophoresed on 0.8% agarose-formaldehyde gel (for *lacZ*Δ mRNA analysis) or 10% acrylamide-7.5 M urea gel (for RNAI), and blotted onto a nylon membrane (23, 24). For probing the *lacZ*Δ RNA, the internal 1.8-kb *Hin*cII fragment of the *lacZ* gene was ^{32}P-labeled with the BRL multiprime labeling kit. RNAI was probed with oligonucleotide 5′-^{32}P-GGATCAAGAGCTACCAACTC-3′ (Fig. 3*a*). 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 *lacZ*Δ 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 P_{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 untranslatable version of the *lacZ* gene (and part of the *lacY* gene) fused downstream of a T7 late promoter (P_{T7}) (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 P_{T7}-*lacZ* construct in ENS32 is described in Fig. 1; the following two points are noteworthy. First, the P_{T7}–*lacZ* junction is so engineered that the high-affinity *lac* repressor-binding site is centered in close proximity to P_{T7} (i.e., at the 13th transcribed nucleotide). This location allows P_{T7} 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 *lacZ*Δ, 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 *lacZ*Δ mRNA appears as a faint smear, with very little material matching the size expected for the full-length transcript (4.2 kb) (Fig. 2*a*, 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. 2*a*; tetracycline experiment is not illustrated). After the addition of any of the drugs, the *lacZ*Δ 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.

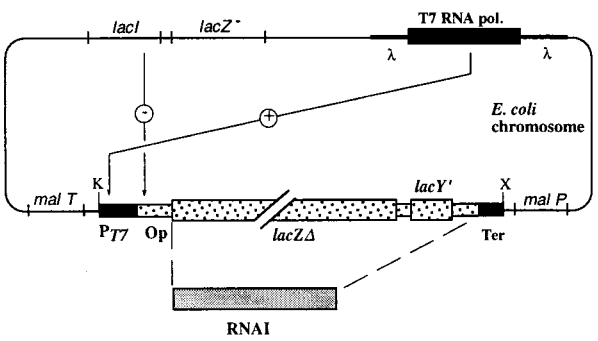

FIG. 1. Schematic representation of the chromosome from the two *E. coli* strains used here. ENS32 harbors an untranslated version of the *lacZ* gene (*lacZ*Δ), and ENS304 harbors a gene encoding RNAI, a down-regulator of ColEI plasmid replication. The black box represents the T7 gene*1*, encoding T7 RNAP. P_{T7} corresponds to the T7 gene*10* promoter from positions −21 to +2 with respect to the natural transcription start; Op (operator) represents the first 22 transcribed nucleotides from the *lac* operon encompassing the high-affinity *lac* repressor-binding site. Ter (terminator) represents the T7 late transcription terminator (Tϕ), fused to the *trp* rho-independent terminator in the case of ENS304 (cf. 19). In ENS32, the operator is followed by the short polylinker from plasmid pEMBLΔ46 (46) and then by a truncated version of the *lac* operon extending from nucleotides +58 to 4010 with respect to the genuine transcriptional start, i.e., lacking the *lacZ* ribosome-binding site. In ENS304, the operator is followed by a *Bam*HI linker and then by the genuine pBR322 RNAI gene (Fig. 3*a*). In both strains, the endogenous *lacZ* gene has been inactivated by Tn*10* insertion (ENS32), followed by imperfect excision (ENS304).

To ascertain this stabilization, we compared directly the decay of the *lacZ*Δ mRNA in ENS32 cells in the absence of drug or after a 20-min kasugamycin treatment. To this end, we switched off rapidly the P_{T7} 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 *lacZ*Δ mRNA was visualized on Northern blots (Fig. 2*b*). In control cells, the faint smear corresponding to the *lacZ*Δ 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 *lacZ*Δ mRNA is not translated, it can be stabilized by translation inhibitors. Moreover, all inhibitors are equivalent in this respect, whether they

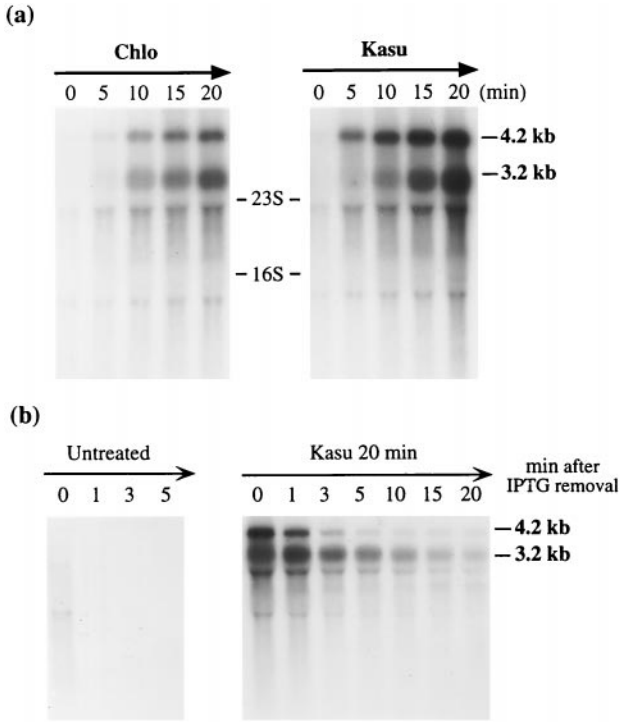

FIG. 2. (*a*) Effect of addition of chloramphenicol (*Left*) or kasugamycin (*Right*) upon accumulation of the *lacZ*Δ mRNA (see *Materials and Methods* for details). At the indicated time after drug addition, the *lacZ*Δ mRNA was visualized in total RNA from ENS32 cells by using Northern blots. The position of the full-length operon transcript (4.2 kb) and of a processed species corresponding to the *lacZ* mRNA proper (3.2 kb), as well as that of the 23S and 16S rRNAs, are indicated. Controls showed that MO00, the parent strain lacking the P_{T7}–*lacZ*Δ construct, produced no *lac*-hybridizing signal under these conditions (cf. 21). (*b*) Northern blot showing the decay of the *lacZ*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*.

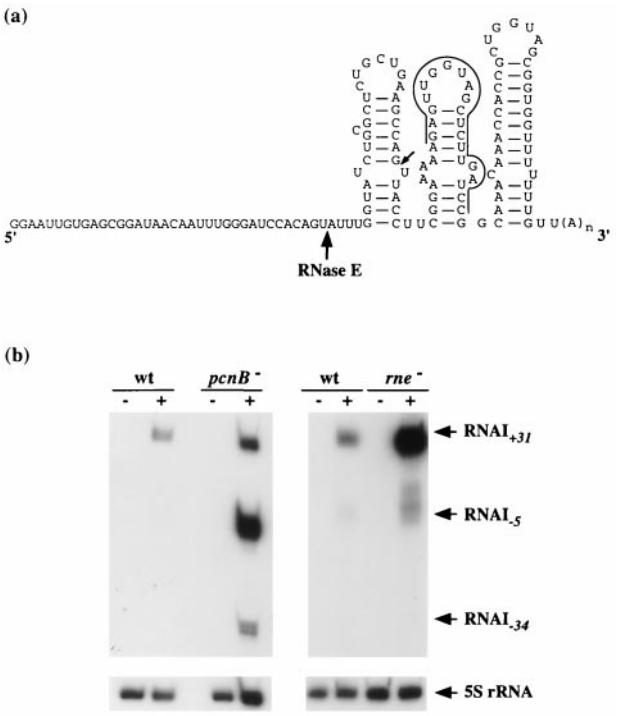

FIG. 3. (*a*) Schematic structure of the engineered RNAI ($RNAI_{+31}$) from ENS304 cells. The three hairpins are tentatively drawn based on the structure of the genuine RNAI. The positions of the major and minor RNase E cleavage sites, generating $RNAI_{-5}$ and $RNAI_{-34}$, respectively, are indicated 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 *pcnB80* derivative (*Left*) or in the wild-type and *rne-1* derivative (*Right*). (*Left*) 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.

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 *lacZ*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 ColE1-derived plasmids, is metabolically unstable, like an mRNA. For consistency, the same genetic system used for the *lacZ*Δ mRNA also was used here. We constructed a strain (ENS304) similar to ENS32 except that the *lacZ*Δ gene is replaced by the RNAI gene from the first nucleotide to the rho-independent terminator (Figs. 1 and 3*a*). In addition, the *lac* operator is still present between the P_{T7} 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. 3*a*). This increase in length can be visualized by primer extension (Fig. 4) by using a probe complementary to the middle of RNAI (Fig. 3*a*). 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 ($RNAI_{-5}$; refs. 11 and 12), which is then rapidly degraded exonucleolytically by polynucleotide phosphorylase (PNPase) assisted by poly(A) polymerase I, the product of the *pcnB* gene (13, 14, 30). In a *pcnB* mutant, the stabilized $RNAI_{-5}$ is slowly processed further by RNase E, yielding in particular a 70-nt species ($RNAI_{-34}$) (15). To check that the P_{T7}-driven RNAI (subsequently named $RNAI_{+31}$ because of its increased length) has a similar decay pathway, total RNA from exponentially growing ENS304 cells was extracted and RNAI-related species were visualized on Northern blots, using the same oligonucleotide probe as above (Fig. 3*a*). Aside from $RNAI_{+31}$, a faint signal corresponding to $RNAI_{-5}$ was observed (Fig. 3*b*, mostly visible on *Right*; Fig. 4). The half-life of $RNAI_{+31}$ species, as estimated from Northern blots after IPTG removal, is *ca.* 2 min (Fig. 5*b*). To ascertain the mechanism of this decay, the *rne-1* mutation, which inactivates RNase E (31), or the *pcnB80* mutations, which inactivate poly(A) polymerase I (22), were introduced into ENS304 cells. The *rne-1* mutation yielded a *ca.* 14-fold accumulation of $RNAI_{+31}$ (at the nonpermissive temperature of 42°C) whereas the *pcnB80* mutation yielded a 150-fold accumulation of $RNAI_{-5}$ (Figs. 3*b* and 4). Interestingly, in either case, the alternate species ($RNAI_{-5}$ and $RNAI_{+31}$, respectively) also accumulated somewhat (Fig. 3*b*), suggesting that RNase E cleavage and subsequent exonucleolytic trimming are interdependent (cf. 14). In addition, RNA_{-34} could be

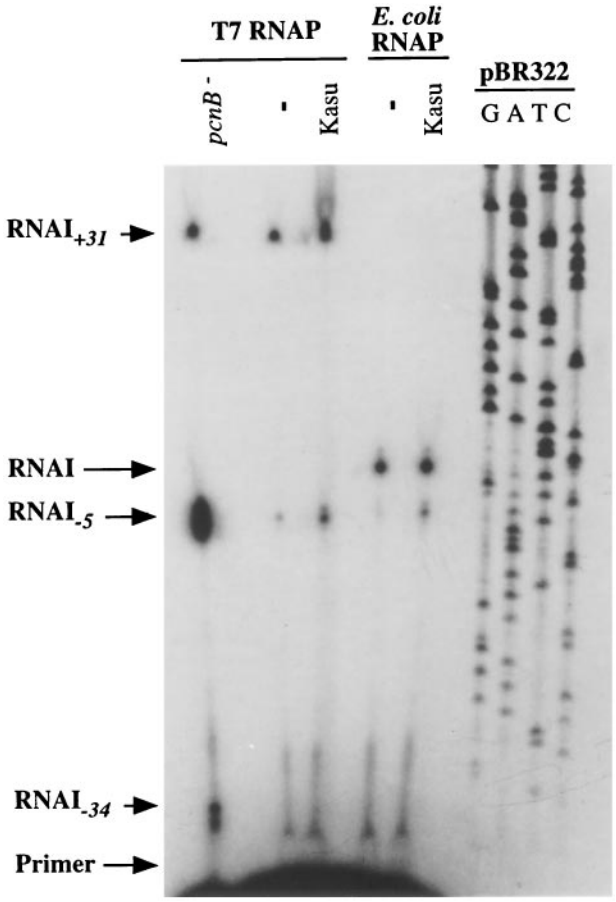

FIG. 4. Primer extension experiment showing the abundance of various RNAI-related species in different cultures. Lanes: 1, strain ENS304, *pcnB80* derivative; 2 and 3, strain ENS304, no treatment or 20-min kasugamycin treatment, respectively; 4 and 5, strain ENS32 harboring plasmid pBR322, no treatment or 20-min kasugamycin treatment, respectively. The primer used is shown on Fig. 3*a*. The sequence lane has been generated from pBR322 with the same primer, allowing the immediate assignment of the different RNAI-related species. Spots corresponding to $RNAI_{+31}$, RNAI, $RNAI_{-5}$, and $RNAI_{-34}$ are indicated by arrows. Note that signals from the single-copy RNAI gene in the ENS304 strain (lanes 2 and 3) match in intensity those generated from the plasmid-borne RNAI gene (lanes 3 and 4), reflecting the unique strength of the P_{T7} promoter (19).

detected in the *pcnB* strain (Figs. 3*b* 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 $RNAI_{+31}$ 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. 5*a*). In the presence of drug, both $RNAI_{+31}$ and $RNAI_{-5}$ accumulated with time. Accumulation was most pronounced for $RNAI_{-5}$, which after 30 min was almost as abundant as $RNAI_{+31}$. The accumulation of $RNAI_{+31}$ and $RNAI_{-5}$ in inhibitor-treated cells is reminiscent of that of the *lacZ*Δ mRNA (Fig. 2*a*). 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 $RNAI_{+31}$ 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 $\ll$1 min, respectively) (Fig. 5*b*).

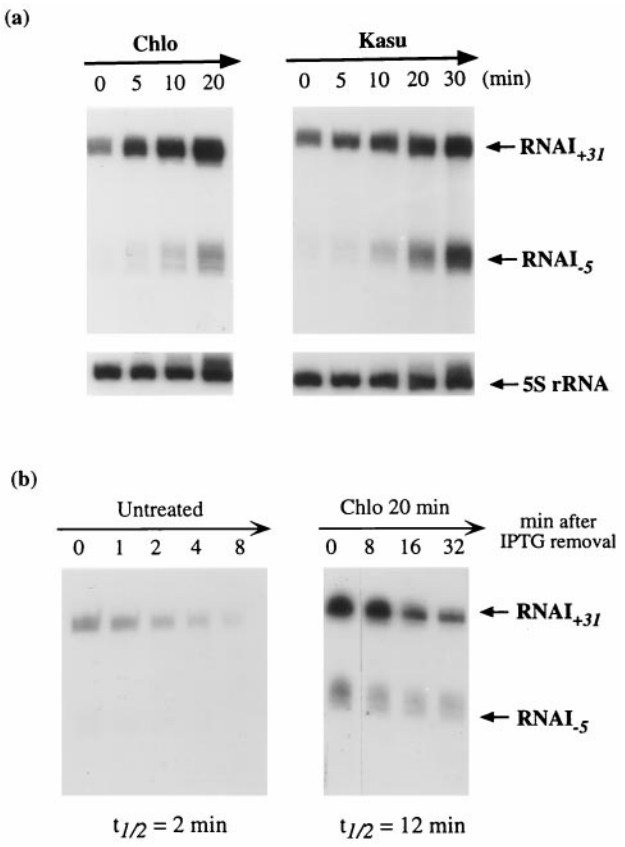

FIG. 5. (*a*) Effect of addition of chloramphenicol (*Left*) or kasugamycin (*Right*) upon accumulation of $RNAI_{+31}$ and $RNAI_{-5}$. At the indicated time after drug addition, total RNA was extracted from ENS304 cells and analyzed on Northern blots. (*b*) Northern blot analysis of the decay of RNA_{+31} and RNA_{-5} in control cells (*Left*) or in cells that have been treated with chloramphenicol for 20 min (*Right*). At zero time, transcription is switched off by transferring the cells into an identical medium lacking IPTG. All symbols are as in Fig. 3*b*.

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 $RNAI_{+31}$ 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. 5*a*). 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 RNA_{-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 *lacZ*Δ mRNA and RNA_{+31} 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. 2*a* with cells overproducing RNase E. RNase E normally autoregulates its own synthesis, but this regulatory loop can be

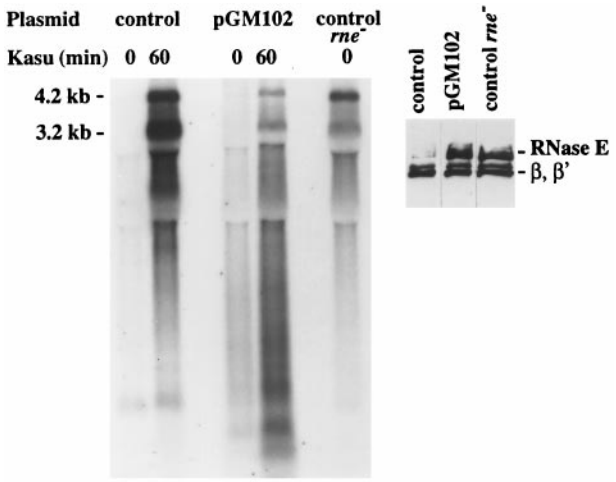

FIG. 6. (*Left*) Northern blot showing the accumulation of *lacZ*Δ mRNA in ENS32 cells harboring either the RNase E-overexpressing plasmid (pGM102) or the parent plasmid pET11a (control). Transcription from the plasmid (and from the *lacZ*Δ gene) was induced 30 min before kasugamycin addition. The RNA 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 *lacZ*Δ mRNA in this strain as compared with the *rne*$^+$ 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 autocontrol.

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 low-molecular-weight species (Fig. 6). This observation suggests that drug treatment slows down not only the RNase E-dependent attack of the *lacZ*Δ mRNA, but also the decay of the resulting fragments. This observation is reminiscent of the stabilization of $RNAII_{-5}$ under these conditions.

DISCUSSION

Drugs That Block Protein Synthesis Stabilize Untranslated mRNAs. We have shown here that three untranslated RNAs, i.e., the *lacZ*Δ mRNA and the $RNAI_{+31}$ and $RNAI_{-5}$ 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 *lacZ*Δ mRNA and $RNAI_{+31}$) and an exonucleolytic process (the PNPase-poly(A) polymerase-mediated degradation of $RNAI_{-5}$) are slowed by the drugs. Moreover, $RNAI_{-5}$ was stabilized to a greater extent than $RNAI_{+31}$, 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 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 *lacZ*Δ mRNA is $\ll$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 *lacZ*Δ 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.

Why Is RNA Stabilized After a Translation Block? The stabilization of the *lacZ*Δ mRNA in drug-treated cells reflects a shortage in RNase E activity under these conditions (Fig. 6). Similarly, the $RNAI_{+31}$ and RNA_{-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.

A 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 $RNAI_{+31}$ or *lacZ*Δ mRNA and the exonucleolytic, PNPase−poly(A) polymerase-dependent degradation of $RNAI_{-5}$ 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 sequestrated 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, rRNA (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 rRNA, because RNase E is responsible for the maturation of 5S rRNA from its immediate precursor, 9S rRNA. Now, when protein synthesis is blocked,

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 *rrn* 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 $RNAI_{-5}$ 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.

Though unproven at this stage, the above interpretation is intriguing because it can account for our observations without the help of ad hoc hypothesis beyond our current knowledge on *E. coli* RNA metabolism. Moreover, it explains simply why the overproduction of RNase E can mitigate the stabilizing effect of translation inhibitors on *lacZ*Δ mRNA (Fig. 6). Were this interpretation correct, then aside from a translation block, other physiological situations that boost rRNA synthesis should also transiently stabilize mRNAs, before the RNase pools reequilibrate. Experimental situations have been described in which the transcription of a plasmid-borne *rrn* operon (or part thereof) is induced (45), so that the cellular concentration of the RNase E target on rRNA increases severalfold. Significantly, we have observed that this induction causes a transient accumulation of both the *lacZ*Δ and *rne* mRNAs. These experiments will be reported elsewhere (I.M., P.J.L., and M.D., unpublished results).

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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