RNase E Cleavage in the atpE Leader Region of atpE/Interferon-β Hybrid Transcripts in Escherichia coli Causes Enhanced Rates of mRNA Decay

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Chimeric transcripts containing the ribosome binding site of the Escherichia coli atpE gene and variants of the human structural interferon-β gene are subject to RNase E processing in the 5′-untranslated atpE part of the transcripts. The absence of processing at two sites in the atpE leader-sequence caused by the RNase E deficiency in E. coli host N3431 leads to a considerable stabilization of the mRNA moiety. RNase E has originally been described as a processing enzyme for non-mRNAs such as precursor 5 S rRNA and RNA1, but cleavage mRNA substrates have also been reported. RNase E processing of the atpE gene leader sequence-containing transcripts leads to an increased rate of mRNA breakdown. The two RNase E-dependent processing sites in the atpE part of the mRNA transcripts exhibit some similarity to the other known RNase E processing sites. The influence of RNase E cleavage upon post-transcriptional regulation such as RNA stability and the efficiency of translational initiation is discussed.

In addition to transcriptional and translational efficiency protein synthesis is controlled by mRNA stability. In this respect mRNA decay rates may vary by as much as 50 among transcripts in a single bacterial cell (Pedersen et al., 1978; Nilsson et al., 1984). In Escherichia coli mRNA stability seems controlled by RNA sequence/structure relationships as well at the 5′, the 3′end, and in addition, also in the interior of (polycistronic) transcripts (Schmeissner et al., 1984; Gorski et al., 1985; Hayashi and Hayashi, 1985; Panayotatos and Truong, 1985; Wong and Chang, 1986; Cannistraro et al., 1986; Newbury et al., 1987; Portier et al., 1987; Cho and Yanofsky, 1988; Chen et al., 1988). Only few of the known RNases seem to be involved in mRNA decay which include 3′exonucleases RNase II and polynucleotidase phosphorylase (Donovan and Kushner, 1986) and endonucleobase RNase III (Dunn and Studier, 1973; Schmeissner et al., 1984; Portier et al., 1987). A conditional lethal mutation in the ans gene exhibits a considerable stabilization of the bulk mRNA in E. coli at the nonpermissive temperature (Ono and Kuwano, 1979). The investigation of rate-limiting steps in mRNA decay has led to the characterization of several enzymes involved in the regulation of mRNA degradation (e.g., Nilsson et al., 1988; Cannistraro and Kennell, 1989). In this respect it also has been demonstrated that RNase E, in addition to the processing of non-mRNA species like RNA1 (Tomcsány and Apirion, 1985) and 5 S rRNA (Apirion, 1978; Ghora and Apirion, 1978), also acts upon T4 gene 32 mRNA thereby converting it into a smaller and considerably more stable mRNA species (Mudd et al., 1988). Recently it has been demonstrated that an E. coli RNase E-deficient background substantially stabilizes bacteriophage T4 mRNAs implying a general role for this endonuclease in mRNA turnover (Mudd et al., 1990).

In a previous study we investigated RNA sequence/structure influences in the translational initiation region of the E. coli atpE gene fused to variants of the human IFN-β gene upon efficiency of translational initiation and mRNA stability (Gross et al., 1990). We observed a correlation between translational efficiency and mRNA stability. In the present investigation we examined the nature of two major endonucleolytic processing sites in the atpE leader sequence upon the decay rates of the hybrid transcripts. Processing in the leader sequence is mediated by endonuclease RNase E cleaving twice the translational initiation region from the E. coli atpE gene. RNase E processing results in enhanced mRNA decay rates of the hybrid transcripts providing further evidence for a role of RNase E in mRNA degradation in E. coli.

EXPERIMENTAL PROCEDURES
Vector Constructions—The expression vector used in this study is pILA-501, which belongs to the family of pILA vectors (Schauder et al., 1987). Bacteriophage λ promoters p8 and p4 are regulated by the temperature-sensitive cl857-coded repressor. Induction is initiated by a temperature shift from 30 to 42 °C. E. coli cells harboring these expression vectors are induced at OD560 = 0.5. The expression vector and the atpE/IFN-β gene hybrid transcripts are as detailed in Fig. 1. Construction and features of atp-IFN, a variant with the primary nucleotide sequence of the natural human IFN-β gene fused to the untranslated atpE leader sequence as well as atp-synIFN, and atp-synIFN variants with the nucleotide sequence of the human IFN-β gene adapted to the optimal E. coli codon usage are as described by Gross et al. (1990). The hybrid transcripts differ in translational initiation rates leading to contrasting IFN-β synthesis in E. coli atp-synIFN represents a variant of the synthetic IFN-β gene with a nucleotide composition in the translational initiation region leading to low level of ribosome binding and a concomitant level of protein synthesis yielding < 1% IFN-β of total E. coli protein. In contrast, variants atp-IFN, and atp-synIFN, exhibit high rates of translational initiation and IFN-β synthesis in E. coli of ~ 18% and 30% of total cellular E. coli protein, respectively.

RNA Kinetics—E. coli cells harboring the expression vectors were grown up to OD560 = 0.5 and induced at 42 °C for 30 min. RIFAMPICIN (150 µg/ml) was added. Cells were taken at the indicated time intervals, quickly pelleted, and frozen at −80 °C in a dry ice bath. RNA was isolated by the guanidinium hot phenol method as described by Sambrook et al. (1989). Total E. coli RNA (5 µg) was separated

Footnotes:
1 The abbreviations used are: IFN, interferon; p8 and p4, early leftward and rightward bacteriophage λ major promoters, respectively.

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electrophoretically in a 2.2 M formaldehyde, 1.5% agarose gel and transferred to nitrocellulose. Hybridization was carried out with a nick-translated 32P-labeled IFN-β gene-specific DNA probe. The half-life of mRNA decay was calculated on the basis of the 32P label present in the intact mRNA band cut from the Northern blot and determined in an scintillation counter. The level of steady-state IFN-β mRNA was determined in the same way by the radioactivity present in the intact IFN-β mRNA band 30 min after induction of transcription.

**Primer Extension Analysis**—Total cellular E. coli RNA (10 μg) or 2 μg of RNA synthesized in vitro by E. coli RNA polymerase from the expression vector (as described by Stüber et al., 1984) was subjected to primer extension analysis by reverse transcription of the human IFN-β gene served with E. coli RNA polymerase and with variants exhibiting high or low rates of translational efficiency (Gross et al., 1987) and exhibits high rates of translational initiation. The variants of the synthetic human IFN-β gene with E. coli codon usage either have reduced or high rates of translational initiation (atp-synIFNβ or atp-synIFNα, respectively). Transcription gives rise to two transcripts from 1,500 and 1,200 nucleotides originating from bacteriophage λ promoters pL and pR. The nucleotide sequence of the translational initiation region is indicated. Major processing sites in this region are denoted by arrows. Bold arrows mark efficient cleavage by endonucleases. The RNase E-dependent cleavage sites at positions 153 and 171 are presented. The position of the initiation 30 S ribosomal subunit is indicated as determined in Gross et al. (1990). Truncated mRNA should only be an ineffective substrate for efficient translational initiation reducing translational efficiency and thereby enhancing the rate of mRNA decay.

**RESULTS**

**mRNA Stability Is Affected by RNase E Cleavage**—In a preceding study we investigated sequence and conformational changes in the translational initiation region upon the efficiency of translational initiation of two IFN-β genes with identical amino acid but differing nucleic acid sequence. One variant represents the natural human IFN-β gene, and the other one was assembled chemically on the basis of the optimal E. coli codon usage. The latter shows a 76% primary sequence identity to the natural human IFN-β gene. Mutagenesis in the translational initiation region of the natural as well as the synthetic IFN-β gene was used to delineate the requirements for optimal translational initiation. Single nucleotide exchanges in this region which altered the mRNA configuration resulted in dramatic changes of IFN-β protein synthesis, rates of translational initiation, and mRNA decay. mRNA stability was correlated with translational efficiency (Gross et al., 1990). Two major endonucleolytic cleavage sites in the untranslated leader region of the pILA-501 (Schauder et al., 1984) was subcloned into the expression vector pILA-501 (Schauder et al., 1987). Deletions in this particular region of the atpE leader sequence impair the efficiency of the ribosome binding site (McCarthy et al., 1985). Therefore, it is likely that processing in the ribosome binding site (Fig. 1; positions 153 and 171) may interfere with efficient ribosome loading and in addition, could be responsible for the observed correlation of translational rates and the rate of mRNA degradation. If this reasoning is correct, the observed processing could constitute rate-limiting steps for mRNA decay in this system. A role of ribosome binding upon mRNA decay rates can also be studied with variants exhibiting high or low rates of translational initiation in RNase-deficient E. coli strains which affect these cleavages.

In this respect we analyzed several E. coli strains with defined RNase deficiencies. E. coli strains with genetic deficiencies in RNases including RNases D, E, I, II, III, and V and polynucleotide phosphorylase were used in this study. Only E. coli strain N3431, characterized by a conditional temperature-sensitive RNase E deficiency, extended the half-life of mRNA breakdown considerably (Fig. 2 and Table I). The experimental system is detailed in Fig. 1. The chimeric IFN-β gene is under the transcriptional control of the two bacteriophage λ promoters pL and pR promoters which give rise to two transcripts from a 1,500 and a 1,200 nucleotides, respectively. E. coli strain N3431 carries a temperature-sensitive mutation inactivating RNase E at 42 °C (Goldblum and Apirion, 1981) which, in this system, is also the temperature of transcriptional induction. The isogenic revertant N3433 shows in
general the rate of mRNA breakdown as observed for E. coli strain DH1 which has routinely been used in the expression studies (Fig. 2 and Table 1). These E. coli strains were used to compare the influence of RNase E processing upon the mRNA half-life of a natural IFN-β gene variant directing a high rate of translational initiation (atp-IFNi) and two synthetic IFN-β gene variants exhibiting low or high rates of translational initiation (atp-synIFN8 and atp-synIFN9, respectively) (Fig. 2 and Table 1). The half-life of atp-IFNi mRNAs transcribed from λ promoters p1 and p2, respectively, in the two transcripts of 1,500 and 1,200 nucleotides are stabilized by a factor of 3.5 or 4, respectively, in E. coli rne- strain N3431 at the nonpermissive temperature (Fig. 2 and Table 1). The isogenic revertant E. coli N3433 exhibits at 42 °C comparable half-lives with E. coli strain DH1 (Fig. 2 and Tab. 1). Similarly, the mRNAs half-lives containing the synthetic IFN-β gene are extended in RNase E-deficient E. coli strain N3431 by a factor of 2.9 in case of the p1 transcript and a factor of 3.7 for the p2 transcript of the atp-synIFN9 variant. In the rne- strain these values for atp-synIFN8 are ~20 and ~16, respectively, because of the short mRNA half-life in rne- E. coli strains (< 0.5 min; Table 1 and Fig. 2).

RNase E Cleavage Takes Place in the Ribosome Binding Site from the E. coli atpE Gene—The major endonucleolytic processing sites in the translational initiation region of atp-IFNi are shown in Fig. 3. Minor cuts take place at positions 138 and 204 and are not caused by RNase E. Another strong signal at position 126 is probably caused by a RNA configuration interfering with the action of reverse transcriptase since this signal could also be detected with in vitro transcribed mRNA (Fig. 3). The cleavage sites at positions 153 and 171 are recognized in rne- E. coli strain DH1 and N3433. They remain uncleaved in E. coli N3431 at the nonpermissive temperature of 42 °C. Its isogenic revertant E. coli N3433 exhibits these cleavage sites and should therefore be product of RNase E processing. In addition, the entire atp-IFNi mRNA moiety was screened for additional RNase E-sensitive sites. They were not observed in either atp-IFNi or in the synthetic IFN-β gene variants atp-synIFN8,9 (data not shown). Therefore, RNase E cleavage at positions 153 and 171 should ultimately be responsible for the differential stability observed for hybrid mRNAs containing the untranslated atpE leader sequence in rne- and rne+ E. coli hosts, respectively.

**Table 1**

| IFN-β gene variants | Level of IFN-β expression in E. coli DH1 | Transcript length originating from bacteriophage λ promoters | mRNA half-life in E. coli | Relative IFN-β mRNA level in E. coli |
|---------------------|----------------------------------------|----------------------------------------------------------|--------------------------|-----------------------------------|
|                     | % total cellular protein | nucleotides | min | DH1 | N3433 (rne- | N3431 (rne+) | DH1 | N3433 (rne-) | N3431 (rne+) |
| atp-IFN1            | 18 | p1 1500 | 3.2 | 3.2 | 11.2 | 15.7 | 18.3 | 43.8 |
|                     |   | p2 1200 | 2.8 | 2.8 | 11.4 | 12.6 | 14.4 | 34.0 |
| atp-synIFN8         | <1 | p1 1500 | 0.5 | 0.5 | 10.3 | 0.7 | 0.9 | 3.8 |
|                     |   | p2 1200 | <0.5 | <0.5 | 8.1 | 0.4 | 0.3 | 1.2 |
| atp-synIFN9         | 28 | p1 1500 | 3.9 | 4.0 | 11.5 | 40.0 | 36.5 | 100.0* |
|                     |   | p2 1200 | 2.3 | 2.2 | 8.5 | 14.4 | 12.7 | 39.8 |
DISCUSSION

This paper demonstrates that processing of atpE leader containing mRNA is dependent on the action of RNase E. An RNase E recognition sequence motif ACAG_{14}AUUUG has been postulated by Tomcsány and Apirion (1985), which together with other RNase E processing sites (Mudd et al., 1988) and the two in the atpE leader sequence reported here seems more as a U-rich sequence flanked by purines: Pu U_{1-4} Pu. Such a degenerate sequence cannot alone define the obviously high cleavage specificity observed for this endoribonuclease. So it seems highly likely that in addition to sequence information RNA configuration confers additive information for processing. However, the region in the atpE leader sequence in which processing takes place is relatively unstructured and does not exhibit a significant stability (~0.7 kcal/mol). Other RNase E processing sites also do not readily denote the secondary structure requirements for efficient RNase E processing (Tomcsány and Apirion, 1985; Mudd et al., 1988).

RNase E cleavage in the upstream region of the E. coli atpE gene results in a significant destabilization of the processed mRNA moiety. One mechanistic model of mRNA decay invokes endonucleolytic cleavages in mRNA as entry sites for the 3' → 5' exonucleases which degrade the resulting mRNA fragments in 3' → 5' direction (reviewed by Belasco and Higgins, 1988). Indeed, mRNA stability of the hybrid transcripts is influenced by 3'-terminal hairpin structures as exhibited e.g. by different terminators of transcription apparently by the capability of the 3' → 5' exonucleases to overcome this RNA configuration (Gross and Hollatz, 1988). In addition, the RNA located 5' upstream of the RNase E cleavage sites could not be detected by Northern analyses and hardly by primer extension analyses, suggesting a high turn-over rate for this particular region (data not shown). This should because RNase E processing creates new 3' ends which serve as entry sites for the 3' → 5' exonucleases. However, another model should be invoked explaining the enhanced decay rates for the RNA located downstream of the processing sites. Ribosome loading in the translational initiation region could interfere with the interaction of endonuclease RNase E with its substrate. A high rate of translational initiation would therefore result in an extended mRNA half-life and in additional rounds of translation. Interference of the initiating ribosomal 30 S subunits with the action of RNase E could explain that RNase E processing is more efficient at position 153 than at position 176, the latter being in closer proximity to the essential features of translational initiation: the Shine-Dalgarno region, the initiator codon AUG and, therefore, to the initiating 30 S ribosomal subunit, although the differential cleavage efficiency could also be the result of RNA sequence/structure relationships at the two cleavage sites. Once RNase E cleavage in the translational initiation region has occurred, ribosome loading could be seriously flawed, leading to a ribosome-depleted mRNA stretch which should exhibit a higher sensitivity toward further endonucleolytic cleavages and an unidirectional net wave of 5' → 3' mRNA degradation as described by Cannistraro et al. (1986) and Belasco and Higgins (1988).

However, another fact adds some complexity to this model. The absence of processing in E. coli N3431 does only marginally increase the steady-state level of atp-synIFN9 mRNA in the E. coli cell. The latter variant exhibits a low rate of translational initiation and a short mRNA half-life of < 0.5 min in rne+ E. coli strains. Although its mRNA is substantially stabilized in an RNase E-deficient strain and about equals the half-life of variants with high rates of translational initiation such as atp-IFN9, or atp-synIFN9 (Table I) this does only result in an increase of the mRNA level by a factor of ~3–4, which is 1–2 orders of magnitude below the steady-state mRNA level observed with variants exhibiting high rates of translational initiation. This could be caused by the transcriptional polarity exerted by E. coli RNA polymerase, which has been demonstrated to take place in a number of cases in which the rate of translational initiation is low (Stanssens et al., 1986; Folley and Yarus, 1989). Otherwise, it seems likely that the initiating 30 S ribosomal subunit rearranges the mRNA configuration (perhaps by local unwinding) so that RNase E is able to recognize and process the transcripts only after interaction with the initiating ribosome. Ribosome binding, i.e. efficient translational initiation, could therefore exert several effects, enabling the access for RNase E processing by local RNA rearrangement and then protection of mRNA by
interfering with mRNA/RNase E interaction. Variants exhibiting a reduced capacity for translational initiation have only a minor fraction of the mRNA population accessible for structural rearrangement induced by 30 S ribosomal subunit binding and, eventually, for RNase E processing. Only this fraction should then be stabilized in an E. coli RNase E deficient strain while the translationally inactive mRNA pool is degraded quickly and efficiently by a mode different from the RNase E-dependent way. In this respect the studies of Chevrier-Miller et al. (1990) should be emphasized; they showed that transcriptional and translational uncoupling leads to differential mRNA half-lives of lacZ mRNA. Their model, according to which the instability of a RNase E mutation obstructs the activity of additional nucleases, could be caused by the absence of protein factor(s) rearranging the local RNA configuration in E. coli as conceived above, although the authors favor a model of a processing enzyme complex in which RNase E is an integral component and in which a RNase E mutation obstructs the activity of additional nucleases.

If RNase E cleavage exerts rate-limiting steps for the stability of actively translated mRNA species this also means that untranslated mRNA is degraded by a mode and by factors that are different and independent from RNase E and which remain to be elucidated.

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REFERENCES

Apirion, D. (1978) Genetics 90, 659–671
Belasco, J., and Higgins, C. (1988) Gene (Amst.) 72, 15–23
Cannistraro, V. J., and Kennell, D. (1989) Eur. J. Biochem. 181, 363–370
Cannistraro, V. J., Subbarao, M. N., and Kennell, D. (1986) J. Mol. Biol. 192, 267–274
Chen, A. C.-Y., Beatty, J. T., Cohen, S. N., and Belasco, J. G. (1988) Cell 52, 609–619
Chevrier-Miller, M., Jacques, N., Raibaud, O., and Dreyfus, M. (1990) Nucleic Acids Res. 18, 5787–5792
Cho, K.-O., and Yanofsky, C. (1988) J. Mol. Biol. 204, 51–60
Donovan, W. P., and Kushner, S. R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 120–124
Dunn, J. J., and Studier, F. W. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2296–2300
Folley, L. S., and Yarus, M. (1989) J. Mol. Biol. 209, 359–378
Ghara, B. K., and Apirion, D. (1978) Cell 15, 1055–1066
Goldblum, K., and Apirion, D. (1981) J. Bacteriol. 146, 129–132
Gorski, K., Roch, J., Prentki, P., and Krisch, H. (1985) Cell 43, 461–469
Gross, G., and Hollatz, I. (1988) Gene (Amst.) 72, 119–128
Gross, G., Mielke, C., Hollatz, I., Blocker, H., and Frank, R. (1990) J. Biol. Chem. 265, 17627–17636
Gurevitz, M., Jain, S. K., and Apirion, D. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4450–4454
Hanahan, D. (1983) J. Mol. Biol. 166, 577–590
Hartz, D., McPeethers, R., Traut, R., and Gold, L. (1988) Methods Enzymol. 164, 419–425
Hayashi, M. N., and Hayashi, M. (1985) Nucleic Acids Res. 13, 5937–5948
McCarthy, J. E. G., Schairer, H., and Sebald, W. (1985) EMBO J. 4, 519–526
Mudd, E. A., Prentki, P., Belin, D., and Krisch, H. M. (1988) EMBO J. 7, 3601–3607
Mudd, E. A., Carponsis, A. J., and Krisch, H. M. (1990) Genes & Dev. 4, 873–881
Newbury, S., Smith, N., Robinson, E., Hiles, I., and Higgins, C. (1987) Cell 48, 297–310
Nilsson, C., Belasco, J. G., Cohen, S. N., and von Gabain, A. (1984) Nature 312, 75–77
Nilsson, C., Lundberg, U., and von Gabain, A. (1988) EMBO J. 7, 2269–2275
Ono, M., and Kuwano, M. (1979) J. Mol. Biol. 129, 343–387
Panayotatos, N., and Truong, K. (1985) Nucleic Acids Res. 13, 2227–2240
Pedersen, S., Reeh, S., and Friesen, J. D. (1978) Mol. Gen. Genet. 166, 329–336
Portier, C., Dondon, L., Grunberg-Manago, M., and Rémige, P. (1987) EMBO J. 6, 2165–2170
Pragai, B., and Apirion, D. (1982) J. Mol. Biol. 154, 465–484
Roy, M. K., Singh, B., Ray, B. K., and Apirion, D. (1983) Eur. J. Biochem. 131, 119–127
Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Schauer, B., Blocker, H., Frank, R., and McCarthy, J. E.-G. (1987) Gene (Amst.) 52, 279–283
Schmeissner, U., McKenney, K., Rosenberg, M., and Court, D. (1984) J. Mol. Biol. 176, 39–53
Stanssens, P., Remaut, E., and Fiers, W. (1986) EMBO J. 5, 3143–3148
Stasovska, T., and Apirion, D. (1985) J. Mol. Biol. 185, 713–720
Wong, H. C., and Chang, S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3233–3237