3′-end processing of precursor M1 RNA by the N-terminal half of RNase E

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Received 21 June 2002; revised 26 August 2002; accepted 27 August 2002

First published online 9 September 2002

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Abstract M1 RNA, the catalytic component of *Escherichia coli* RNase P, is derived from the 3′-end processing of precursor M1 RNA, a major transcript of the *rnpB* gene. In this study, we investigated the mechanism of 3′-end processing of M1 RNA using the recombinant N-terminal half RNase E. The cleavage site preference of RNase E differed from that of the 40% ammonium sulfate precipitate (ASP-40), a partially purified cell extract containing processing activity. However, the addition of a trace amount of ASP-40 changed the cleavage site preference of RNase E to that of ASP-40 suggesting the involvement of a soluble factor in cleavage site preference.

Key words: RNase E; N-terminal half; M1 RNA; 3′ processing; *Escherichia coli*

1. Introduction

RNase E, an essential endonuclease of 1061 amino acids, was initially characterized as the enzyme that processes 9S rRNA to yield 5S rRNA in *Escherichia coli* [1]. Recently, RNase E was shown to be the main component of the mRNA degradation machinery (degradosome) [2–7]. The N-terminal half (NTH) of RNase E contains the catalytic site for its single-strand-specific endonuclease activity, while the C-terminal half of the enzyme functions as a scaffold for degradosome formation [8–12]. It has been reported that the C-terminal half of RNase E is instrumental in degrading mRNA, but dispensable for processing rRNA [13,14]. Recent studies also have shown that RNase E is required for the initiation of tRNA maturation and that this function is essential role of RNase E for the cell viability [15,16].

Another endonuclease, RNase P, is a tRNA processing enzyme responsible for cleaving the 5′-leader sequence to generate the mature 5′-end of tRNA [17,18]. *E. coli* RNase P contains a 377 nucleotide RNA called M1, which functions as the catalytic subunit of the enzyme and is formed through a processing event. M1 RNA is transcribed from the *rnpB* gene as a precursor molecule called pM1 RNA [18–20], which is processed at the 3′-end to generate the mature M1 RNA [21–25]. Although the processing activity has not been fully characterized, several studies have shown that it is related to RNase E activity. Gurevitz et al. [22] have shown that pM1 RNA accumulates at non-permissive temperatures in a temperature-sensitive RNase E mutant strain. However, they found that partially purified RNase E does not catalyze this processing event in vitro. Therefore, they postulated that RNase E is indirectly involved in the processing. However, Lundberg and Altman [23] reported that RNase E activity correlates with the 3′-end processing activity of M1 RNA, suggesting the direct involvement of RNase E. This notion is supported by the presence of a pentanucleotide sequence immediately adjacent to the processing site, GAUUU, that matches the consensus sequence of the RNase E cleavage site (the *rne*-dependent site) (Fig. 1; [26]). Alteration of the GAUUU sequence affects the processing efficiency in vivo and in vitro [24,27] lending further support for the direct involvement of RNase E. Furthermore, 3′-end processing of M1 RNA is a complex process involving two multi-step pathways [28]. So it is still unclear how RNase E activity is involved in the processing of M1 RNA.

In this study, we examined the 3′-end processing of M1 RNA in vitro using the N-terminal catalytic half of RNase E. The results suggest that RNase E directly participates in one of the processing pathways (pathway II), and is also involved in the second step of the other processing pathway (pathway I). Interestingly, the cleavage site preference of RNase E in pathway II was different from that of the 40% ammonium sulfate precipitate (ASP-40), a partially purified cell extract containing processing activity. However, this cleavage site preference can be changed to that of ASP-40 by the addition of a trace amount of ASP-40 suggesting the involvement of a soluble factor in cleavage site preference.

2. Materials and methods

2.1. Bacterial strains and plasmids

E. coli JM109 [29] was used for the construction of plasmids and the preparation of cell extract. The expression vector pET21b from Novagene was used for expression of the recombinant RNase E. Plasmid pSPd23 contains the *rnpB* transcription unit with an internal deletion of the sequence between +57 and +330, and carries the SP6 promoter linked to the 5′-end of the M1 RNA-coding sequence and a *Dra*I site at position +415 [24]. pSPM1p is also a derivative of pALTER-1, which was equivalent to pSPd23 but having the intact *rnpB* sequence to generate pM1 RNA [28].

2.2. Expression and purification of the recombinant RNase E and RNase G

For construction of pRNEN, an expression plasmid for the His-tagged NTH of RNase E, the 1584-bp fragment encoding the N-terminal 528 residues of the *rne* gene was generated by PCR amplification of the corresponding region of *E. coli* chromosomal DNA with

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0014-5793/02/$22.00
PII: S0014-5793(02)03345-8

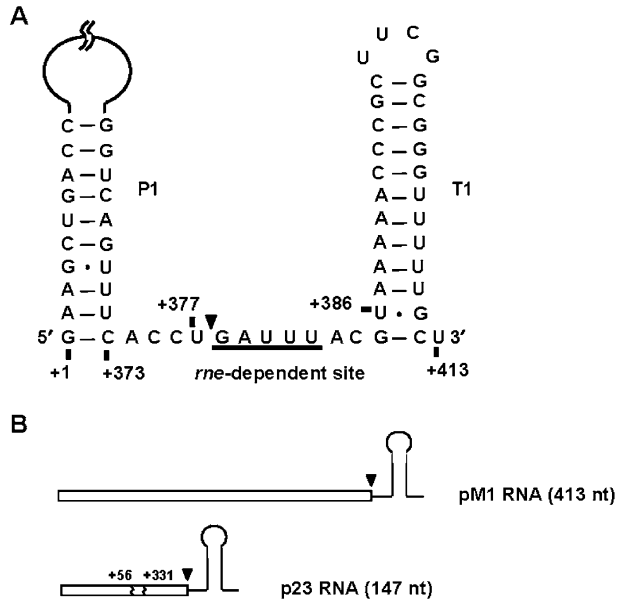

Fig. 1. A: The secondary structure of M1 RNA surrounding the processing site. The arrowhead indicates the 3′-end of the mature M1 RNA. The *rne*-dependent site is underlined. B: Schematic representation of pM1 RNA and p23 RNA. The rectangle indicates the structural sequence of M1 RNA. The internal deletion points in p23 RNA are indicated by wavy lines and numbers, at which an *Eco*RI linker sequence of 8 nucleotides was inserted [24]. The transcription terminator is indicated by the stem–loop structure.

the oligonucleotide primer pairs 5′-CCGGATCCGATGAAAA-GAATGTTAATCAA-3′ and 5′-GTCTCGAGCGCAGGTTGTTCC-3′. The PCR product was cut with *Bam*HI and *Xho*I, and cloned into pET21b. The NTH of RNase E was overexpressed in *E. coli* BL21(DE3) cells containing pRNEN. Cells were grown exponentially in Luria–Bertani medium containing ampicillin (50 μg/ml) at 30°C with shaking. At an A_{600} of 0.5–0.6, isopropyl-D-thiogalactoside was added to a final concentration of 1 mM, and the cultures were incubated for an additional 2 h. The cells were collected by centrifugation at 4°C. The pelleted cells were resuspended in column buffer (10 mM Tris–HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA) and sonicated on ice. After sonication, the supernatant fraction was subjected to Ni-NTA affinity chromatography as described in the pET System Manual (Novagen). The protein preparation was concentrated after dialysis against 50 mM Tris–HCl, pH 7.5, 20% glycerol, 1 mM EDTA, 500 mM NaCl and 0.5% Triton X-100. The concentration of the protein was determined by the BCA method [30].

To construct pRNG, an expression plasmid for the His-tagged RNase G, we amplified the *rng* coding region with the primer pairs 5′-GCCCGGGCATATGACGGCTGAATTGTTAGTAAACG-3′ and 5′-GCGCTCGAGCATCATTACGACGTCAAACTGCTCC-3′. The PCR product was cut with *Nde*I and *Xho*I, and cloned into pET21a. The recombinant RNase G was overexpressed and purified by the same methods as described above for the NTH-RNase E.

2.3. In vitro transcription of substrates

The plasmids pSPd23 and pSPM1p were cleaved with *Dra*I for the generation of p23 RNA and pM1 RNA, respectively, and used as templates for in vitro transcription by SP6 RNA polymerase [27]. A DNA template for transcripts having a 3′-end of +386 was constructed as described [27]. In vitro transcripts were labeled at the 3′-end with [^{32}P]pCp and T4 RNA ligase, or internally with [α-^{32}P]CTP, as described [31]. RNA products were purified before use by gel elution, as described [31].

2.4. In vitro processing assay

The ASP-40 of the S30 fraction was prepared as a partially purified cell extract from *E. coli* JM109, as described [24]. RNA substrates of 0.2 pmol were incubated with the ASP-40 fraction or the recombinant protein at 37°C in 50 mM Tris–HCl, pH 7.5, 60 mM NH_4Cl, 10 mM $MgCl_2$, 0.5 mM DTT, and 0.1 mM phenylmethylsulfonyl fluoride, as described [24]. Yeast tRNA at a concentration of 0.7 mg/ml was added into the reaction mixture when both the ASP-40 fraction and the recombinant RNase E were included in the same reaction. Yeast total RNA (0.7 mg/ml) was added in the reaction mixture when required. The reaction products were extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and analyzed by electrophoresis on a polyacrylamide sequencing gel containing 8 M urea. The cleavage preference was calculated as a ratio of the amount of each processed RNA to that of the total processed RNA as determined by an image analyzer BAS-1500 (Fuji).

2.5. Gel permeability chromatography (GPC)

The S30 fraction which was prepared from *E. coli* JM109 as described [24], and the affinity-purified NTH-RNase E were applied to a FPLC system with HiLoad 16/60 Superdex chromatography (Pharmacia). The flow rate was kept at 1 ml/min with buffer A containing 50 mM Tris–HCl, pH 7.5, 60 mM NH_4Cl, 10 mM $MgCl_2$, and 0.5 mM

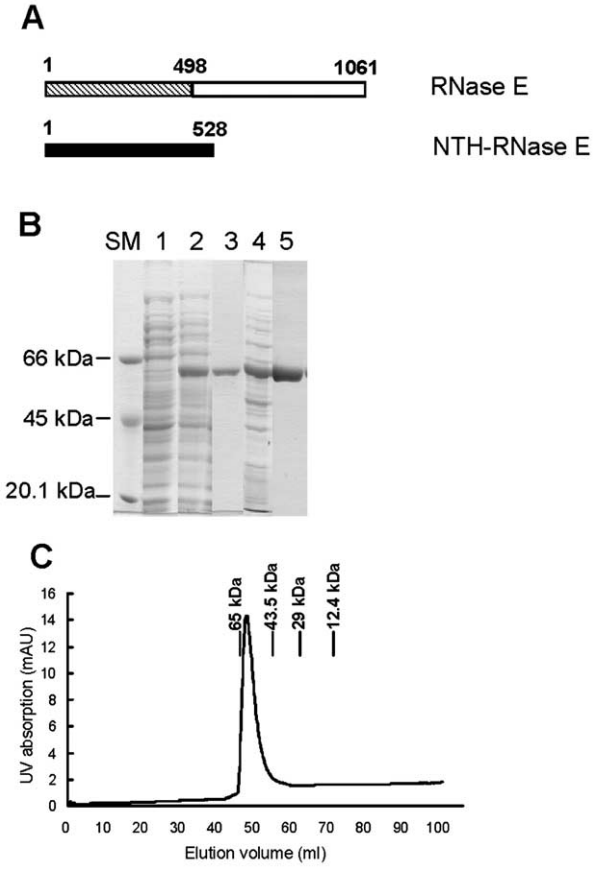

Fig. 2. Preparation of the recombinant RNase E and RNase G. A: Structure of RNase E. The minimal NTH for retaining endonuclease activity [10] is indicated by the hatched rectangle. The solid bar indicates the NTH used for the preparation of the recombinant RNase E. B: *E. coli* BL21(DE3) cells containing pRNEN were grown and induced with 1 mM IPTG. The expressed recombinant RNase E as a His-tagged fusion protein was purified by Ni-NTA affinity chromatography. The protein samples were analyzed on a 10% SDS–polyacrylamide gel. Lane 1, total proteins of the uninduced *E. coli* cells; lane 2, total proteins of the IPTG-induced *E. coli* cells; lane 3, the purified recombinant RNase E (1 μg); lane 4, total proteins of *E. coli* cells overexpressing His-tag fused RNase G; lane 5, the purified recombinant RNase G (5 μg). SM, size markers. C: Purification of NTH-RNase E by GPC. The Ni-NTA affinity-purified NTH-RNase E was subjected to GPC. Ultraviolet absorption of eluants was monitored. Elution points of standard proteins were indicated on the elution profile.

DTT for the whole time of the run. Two milliliter fractions were collected.

3. Results

3.1. In vitro processing of pM1 RNA with recombinant RNase E

We prepared the recombinant NTH-RNase E as a fusion protein tagged at the C-terminus with six histidine residues. The protein was purified by Ni-NTA affinity chromatography as a single band on an SDS–polyacrylamide gel (Fig. 2).

The NTH-RNase E was used for the in vitro cleavage reaction of pM1 RNA. Indeed, the recombinant NTH-RNase E cleaved pM1 RNA at the expected processing site (Fig. 3A). However, it was very difficult to determine the cleavage site at the nucleotide level because pM1 RNA is a relatively long RNA molecule. Therefore, we also investigated the processing of p23 RNA, a truncated pM1 RNA by NTH-RNase E (Fig. 3B). This truncated RNA has been successfully used as a model substrate for the study of 3′-end processing of M1 RNA both in vivo and in vitro [24,27,28], and provided a more accurate determination of the cleavage specificity at single nucleotide resolution. p23 RNA was cleaved by NTH-RNase E to yield exclusively the processing product (termed +379 RNA) having the 3′-ends of +379 (90% preference over +378 RNA). This cleavage site preference differed from that of ASP-40 as a partially purified cell extract containing processing activity. ASP-40 generated the processing products (termed +378/+379 RNA) having the 3′-ends of +378 and +379 with the preference of the 3′-end of +378 (60% preference). The error was generally 10% or less. These results were confirmed by repeating the experiment several times using different extract preparations. To determine whether both cleavage reactions occurred by endonucleolytic mechanisms, the substrates were 3′-end labeled with ^{32}P and assessed in the processing assay (Fig. 4). The 3′-cleavage fragments matched their cleavage site preference in the processing reaction with the internally labeled substrates. Actually, the corresponding 3′-cleavage products from the internally labeled substrate were also observed with a relatively short running time on a 10% polyacrylamide gel (data not shown), as shown elsewhere [28]. pM1 RNA was also labeled at the 3′-end and used as a substrate for the cleavage reaction. The difference in cleavage site preference between NTH-RNase E and ASP-40 was the same as that for p23 RNA. The matching of the cleavage site preference between the processing reactions with the internally and 3′-end-labeled substrates indicates that the cleavage site preference is the result of endonucleolytic cleavage by both NTH-RNase E and ASP-40, and not the result of 3′-end trimming by an exoribonuclease activity following the endonucleolytic cleavage reaction.

If another endonuclease is responsible for the +378 cleav-

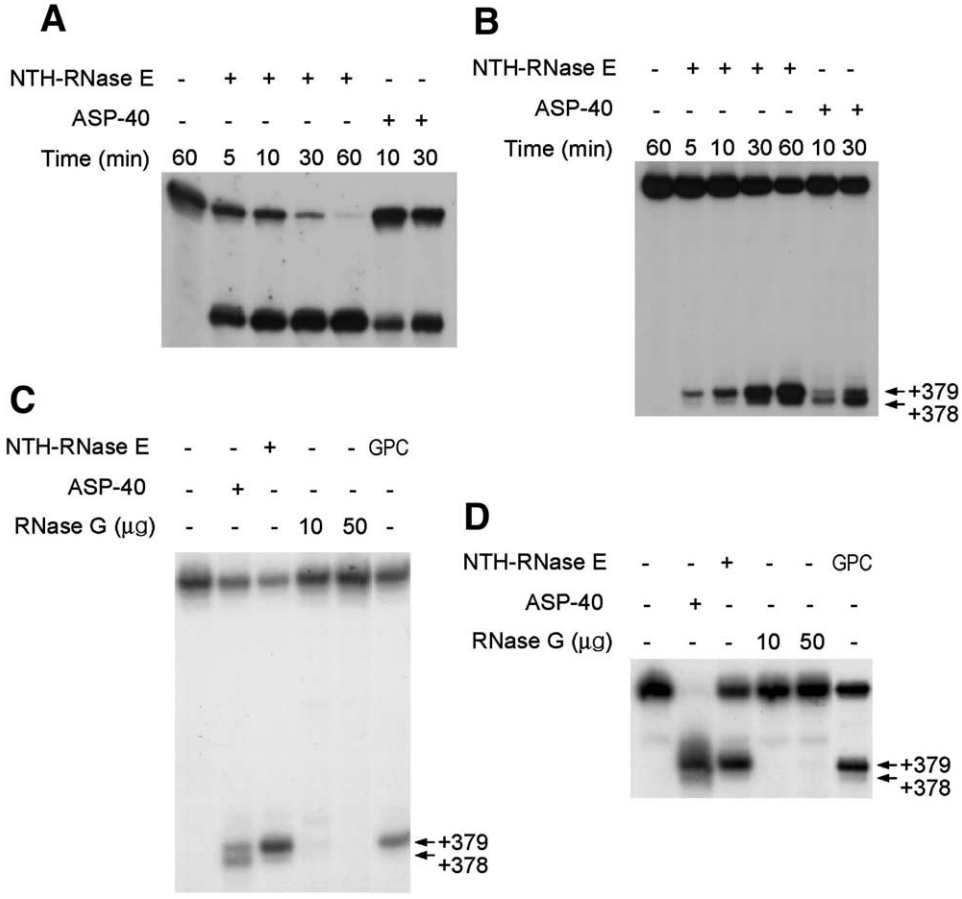

Fig. 3. In vitro processing reactions. Substrate RNA molecules, pM1 RNA (A), p23 RNA (B, C), and +386 intermediate RNA (D) were internally labeled with [α-^{32}P]CTP. The labeled RNA substrates were incubated at 37°C with ASP-40 (2 μg of protein), with 10 ng of NTH-RNase E, or the indicated amounts of RNase G. In vitro reaction products were electrophoresed on 5% polyacrylamide sequencing gels. GPC stands for the NTH-RNase E protein that was further purified by GPC.

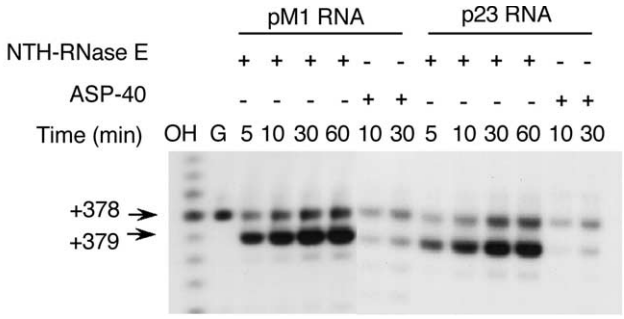

Fig. 4. Identification of downstream cleavage products. RNA substrates labeled at the 3′-end with [^{32}P]pCp were incubated with 10 ng of NTH-RNase E or ASP-40 (2 μg of protein) and analyzed on a 10% polyacrylamide sequencing gel. OH indicates alkaline hydrolysis ladders. G stands for G-specific (RNase T1) cleavage products.

age, RNase G would be a candidate since it is homologous to the N-terminal catalytic domain of RNase E [32]. Therefore, we tested whether RNase G could cleave p23 RNA. However, the recombinant RNase G as a His-tag fused protein did not generate any cleavage products (Fig. 3C), while it was active on the RNA I substrate (data not shown).

ASP-40 also generates additional products (termed +385/+386 RNA) with the 3′-ends of +385 and +386, which are further processed to +378/+379 RNA [28]. +385/+386 RNA intermediates are usually not observed because ASP-40 rapidly processes them [24]. However, these RNA intermediates accumulate in the presence of excess yeast total RNA which inhibits their further processing [24,28]. Currently, it is not known whether RNase E was responsible for the generation of +385/+386 intermediates. As shown in Fig. 3, the recombinant NTH-RNase E did not generate +385/+386 intermediates not even in the presence of yeast total RNA (data not shown). These results suggest that RNase E is involved in 3′-end processing of M1 RNA through pathway II by endonucleolytically generating +379 RNA, and not through pathway I by generating +385/+386 intermediates.

The processing of the +386 intermediate to +378/+379 RNA, which is the second step of pathway I, has been thought to be *rne*-independent because this step occurs with ASP-40 isolated from *rne*-3071 mutant cells. Since the intermediate RNA was also cleaved by NTH-RNase E (Fig. 3D), multiple enzymes seem to be involved in the processing of +385/+386 RNA to +378/+379 RNA. However, RNase G does not seem to be responsible for this process because RNase G did not cleave the intermediate RNA (Fig. 3D, lanes 4 and 5).

3.2. The presence of a cellular factor alters cleavage specificity by NTH-RNase E

We examined whether the difference of the cleavage preference between NTH-RNase E and ASP-40 arises from the lack of the C-terminal half of RNase E or from the presence of a cellular factor in ASP-40. For this purpose, we carried out the processing reaction by NTH-RNase E in the presence of ASP-40 (Fig. 5). The addition of ASP-40 inhibited the processing activity of NTH-RNase E, probably due to the presence of large amounts of RNA in ASP-40. To reduce the inhibition effect of ASP-40, we added excess yeast tRNA to the reaction. However, because the additional yeast tRNA also inhibited NTH-RNase E activity, we instead used a large amount of NTH-RNase E in the processing reactions. We first varied the amount of NTH-RNase E in the in vitro processing assay with 2 μg of ASP-40. This amount of ASP-40 alone generated a very small amount of products, but changed the cleavage site preference of NTH-RNase E to that of ASP-40 (Fig. 5A). The generation of products with the changed cleavage site preference increased with the amount of NTH-RNase E that was added to the processing reaction, indicating that the cleaving activity with the change of the cleavage site preference originated from NTH-RNase E and not from ASP-40. This means that the change in the cleavage site preference was not due to the lack of the C-terminal half of RNase E. Alternatively, we varied the amount ASP-40 in the in vitro processing assay with a fixed amount of NTH-RNase E (Fig. 5A). Interestingly, a trace amount of ASP-40 was sufficient to change the cleavage site preference of NTH-RNase E. These results suggest there might be a factor or factors in ASP-40 that can alter cleavage site specificity. We also examined whether the addition of ASP-40 would modify the NTH-

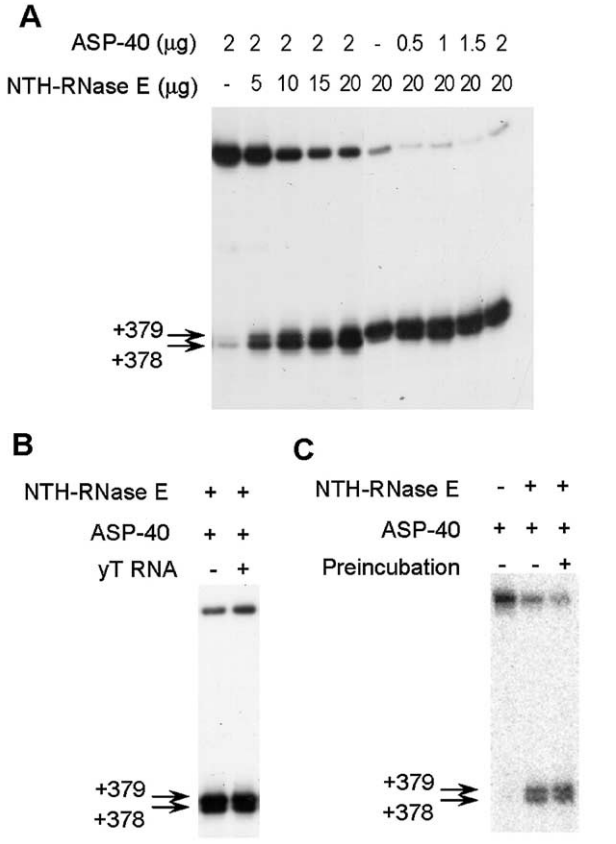

Fig. 5. Alteration of the cleavage preference of NTH-RNase E in the presence of ASP-40. A: p23 RNA was internally labeled with [α-^{32}P]CTP. The labeled RNA was incubated with increasing amounts of NTH-RNase E (5–20 μg) in the presence of the fixed amount of ASP-40 (2 μg of protein) at 37°C for 5 min. Alternatively, the labeled RNA was incubated with increasing amounts of ASP-40 (0.5–2 μg of protein) at the fixed amount of NTH-RNase E (20 μg). B: The labeled p23 RNA was incubated with 20 μg of RNase E in the presence of ASP-40 (0.5 μg of protein). The presence and absence of excess yeast total RNA (yT RNA) in the reaction mixture are indicated as plus (+) and minus (−). C: The labeled p23 RNA was added after the preincubation of ASP-40 (0.5 μg of protein) with 5 μg of NTH-RNase E for 5 min.

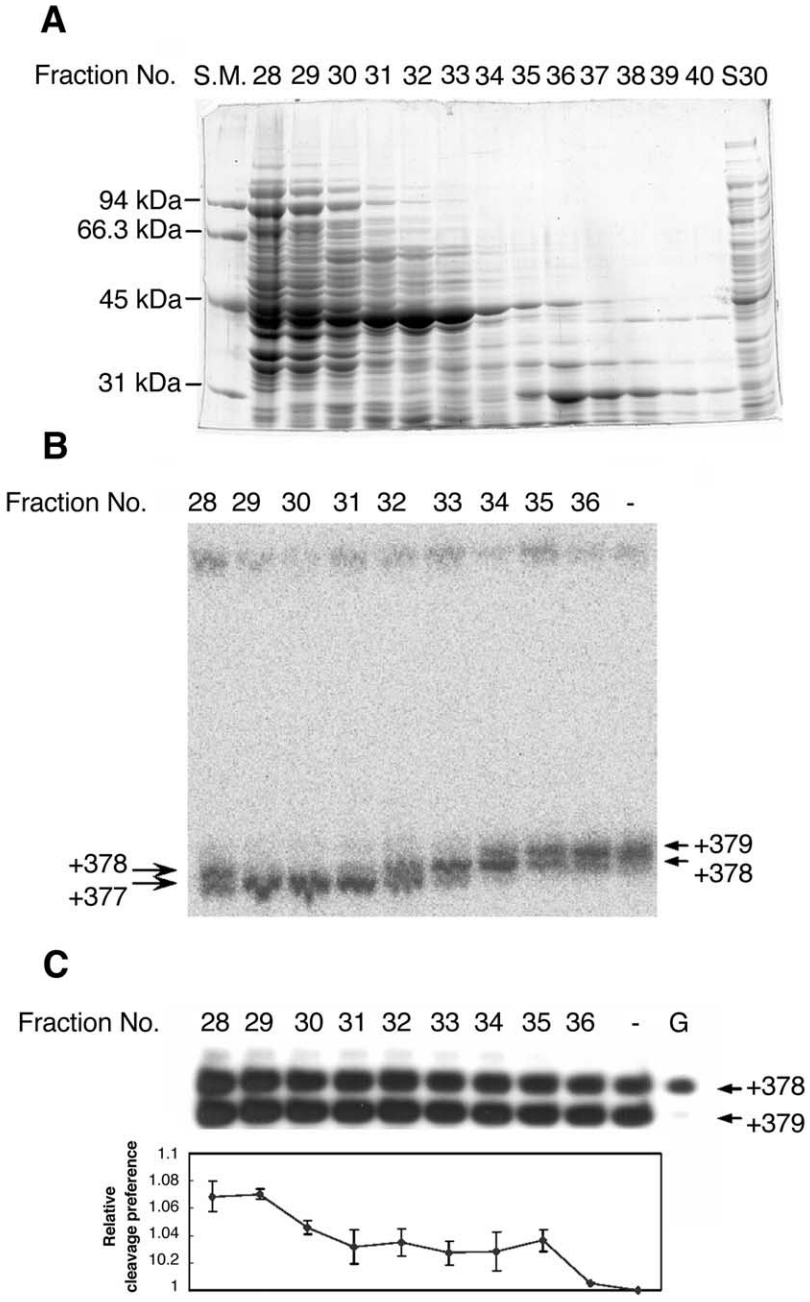

Fig. 6. Alteration of the cleavage preference of NTH-RNase E by fractions of the S30 cell extract separated through GPC. A: Fractions were analyzed on a 10% SDS–polyacrylamide gel. The internally labeled (B) or the 3′-end labeled (C) p23 RNA substrate was incubated with 100 ng of RNase E in the presence of 10 μl of each fraction at 37°C for 5 min. The reaction products were analyzed on 5% or 10% polyacrylamide sequencing gels. G stands for G-specific (RNase T1) cleavage products of the substrate. The relative cleavage preference represents a relative increase of the +378 cleavage product by the addition of each fraction.

RNase E activity to produce the +385/+386 RNA intermediates. In this experiment, we included excess yeast total RNA in the in vitro processing assay to exclude the possibility that +385/+386 RNA could be further processed by the added ASP-40 [28]. The addition of ASP-40 did not induce NTH-RNase E to generate the +385/+386 RNA intermediates (Fig. 5B).

One may still argue that the cleavage at the +378 position is produced by an activity in the ASP-40. This argument comes from the possible limitation of cleavage of the p23 RNA by the enzyme within the ASP-40: this enzyme activity might be competitively inhibited by some RNA molecules that are included in the ASP-40. The addition of NTH-RNase E would result in the preferential decay of any competitive inhibitor RNA, thereby enhancing the +378 cleavage. To test this possibility, we preincubated ASP-40 with NTH-RNase E before adding the substrate so that any competitive inhibitor RNA would be degraded. However, we did not find any enhancing effect on the +378 cleavage by this preincubation (Fig. 5C). Therefore, no enzyme other than NTH-RNase E seems to be responsible for the +378 cleavage.

With a purpose to identify the factor affecting the cleavage

specificity of NTH-RNase E, we tried to separate proteins included in the ASP-40 according to their size by GPC. Because the separating condition was non-denaturing, most of the proteins were obtained in the form of complexes (Fig. 6A). When the processing activity of each fraction was tested, the processing activity was isolated with fractions 29, 30, and 31, which were eluted at about 60 min, showing the majority of the processing activity (data not shown). We examined the effect of each fraction on the cleavage preference of NTH-RNase E by adding a trace amount of the fraction in the processing reaction. Fractions 33–35 changed the cleavage preference of NTH-RNase E from position +379 to +378 (Fig. 6B). Fractions 28–33 caused the generation of the +377 cleavage product in addition to the +378 cleavage product. To assess whether the +377 and +378 cleavage products were generated by endonucleolytic cleavage reaction or by a trimming reaction mediated by exoribonucleases, the 3′-end-labeled substrate was used in the same processing assay. The 3′-end cleavage fragment by endonucleolytic cleavage at the +378 site slightly increased with the addition of fractions 33–35. This increase was not as much as that shown with the internal labeled substrate. These data suggest that fractions 33–35 carry an exoribonuclease that is responsible for one-nucleotide trimming at the 3′-end, in addition to the factors that affect the cleavage preference of NTH-RNase E. In contrast, the 3′-endonucleolytic fragment corresponding to the +377 cleavage that occurred in the presence of fractions 28–33 was not observed. Therefore, fractions 28–33 must contain an exoribonuclease that catalyzes the trimming reaction to generate the 3′-end of +377. This result confirms that the +377 RNA is formed after the initial endonucleolytic cleavage by trimming one- or two-nucleotides at the 3′-end [33].

4. Discussion

In this study, we showed the direct involvement of RNase E in the 3′-end processing of M1 RNA using NTH-RNase E as the catalytic domain of RNase E. NTH-RNase E cleaves exclusively the phosphodiester bond after the +379 nucleotide. One may argue that this processing activity would be due to *E. coli* proteins copurified with NTH-RNase E during the purification by Ni-NTA affinity chromatography. To exclude this possibility, we further purified NTH-RNase E by GPC. The GPC-purified NTH-RNase E, which was eluted as a single peak of about 60 kDa (Fig. 2C), did not show any contaminating protein band in a silver-staining SDS–polyacrylamide gel (data not shown). The processing activity of the GPC-purified NTH-RNase E was the same as that of NTH-RNase E purified from the Ni-NTA affinity column (Fig. 3C,D). The cleavage site by NTH-RNase E differs from the cleavage site preference of ASP-40, a partially purified cell extract that cleaves the phosphodiester bonds after nucleotide positions +378 and +379 with a cleavage site preference at position +378. This cleavage site preference was changed to that of ASP-40 by the addition of a trace amount of ASP-40 in the NTH-RNase E reaction. This result suggests that the change in the cleavage site preference originated from the presence of a cellular factor in ASP-40, rather than the lack of the C-terminal half of RNase E in NTH-RNase E. Although the identity of this putative cellular factor is unknown, it would have to be a factor that could interact with the RNA substrate or the N-terminal region of RNase E to alter the cleavage specificity of RNase E.

Why is the cleavage specificity of RNase E changed by a cellular factor? One possible explanation would be that cleavage specificity is controlled by various cellular factors in vivo. In this respect, it is noteworthy that RNase E is not only an RNA processing enzyme but also a core enzyme of the general RNA degradation machinery (degradosome) [2–7]. A single nucleotide change at the 3′-end may cause a significant change in the stability of the RNA or in its processing. It is not known at this time whether the two RNA molecules having different 3′-ends of +378 and +379 are processed through different pathways. It may be possible since the two nucleotide difference in length at the 3′-end of pM1 RNA itself determines by which processing pathway it will follow [28]. If this was the case, the change of the cleavage site specificity would be a prerequisite for the function of RNase E as a processing enzyme.

When the S30 cell extract was fractionated using a size-exclusion column, we observed the change in the cleavage site by NTH-RNase E with the addition of some fractions. However, the cleavage products were produced not only by the change of the endonucleolytic cleavage site, but also by the action of exoribonucleases. This exoribonuclease activity was not observed in the ASP-40 or the S30 fraction [27,28]. Exoribonuclease activities may normally be tightly regulated in the cell, since the 3′-end of RNA can be an important signal for the destination of RNA [28,34]. Therefore, the appearance of the exoribonuclease activity in size exclusion fractions of S30 might result from disruption of the normal regulation in the cell by fractionation of the cell extract.

We previously showed that 3′-end processing of M1 RNA occurs through two processing pathways [27,28]. In pathway I, pM1 RNA is cleaved to generate the +385/+386 RNA intermediates (the first step), which are subsequently processed to generate +378/+379 RNA (the second step). Experiments with cell extracts derived from an *E. coli* strain carrying the *rne*-3071 mutation responsible for thermosensitivity of RNase E function showed that the second step is *rne*-independent [28]. However, in this paper, we showed that the purified NTH-RNase E could cleave the intermediate RNA at the expected site. These data suggest that the second step of pathway I can occur by multiple enzymes including RNase E. The *rne*-dependency of the first step of pathway I was unclear because this step itself was temperature-dependent. In pathway II, pM1 RNA is directly processed to generate +378/+379 RNA and this step is *rne*-dependent [28]. NTH-RNase E generated +379 RNA by an endonucleolytic cleavage, but it did not generate the +385/+386 RNA intermediates (Figs. 3 and 5). Therefore, RNase E does not seem to be involved in the generation of the +385/+386 intermediates.

Acknowledgements: This work was supported by Korea Research Foundation Grant KRF-2000-015-DP0293.

References

[1] Ghora, B.K. and Apirion, D. (1978) Cell 15, 1055–1066.
[2] Carpousis, A.J., Van Houwe, G., Ehretsmann, C. and Krisch, H. (1994) Cell 76, 889–900.
[3] Py, B., Causton, H., Mudd, E.A. and Higgins, C.F. (1994) Mol. Microbiol. 14, 717–729.
[4] Py, B., Higgins, C.G., Krisch, H.M. and Carpousis, A.J. (1996) Nature 381, 169–172.

[5] Miczak, A., Kaberdin, V.R., Wei, C.-L. and Lin-Chao, S. (1996) Proc. Natl. Acad. Sci. USA 93, 3865–3869.
[6] Rauhut, R. and Klug, G. (1999) FEMS Microbiol. Rev. 23, 353–370.
[7] Coburn, G.A. and Mackie, G.A. (1999) Prog. Nucleic Acid Res. Mol. Biol. 62, 55–108.
[8] Taraseviciene, L., Bjork, G.R. and Uhlin, B.E. (1995) J. Biol. Chem. 270, 25391–26398.
[9] Kido, M., Yamanaka, K., Mitani, T., Niki, H., Ogura, T. and Hiraga, S. (1996) J. Bacteriol. 178, 3917–3925.
[10] McDowall, K.J. and Cohen, S.N. (1996) J. Mol. Biol. 255, 349–355.
[11] Kaberdin, V.R., Miczak, A., Jakobsen, J.S., Lin-Chao, S., McDowall, K.J. and von Gabain, A. (1998) Proc. Natl. Acad. Sci. USA 95, 11637–11642.
[12] Vanzo, N.F., Li, Y.S., Py, B., Blum, E., Higgins, C.F., Raynal, L.C., Krisch, H.M. and Carpousis, A.J. (1998) Genes Dev. 12, 2770–2781.
[13] Ow, M.C., Liu, Q. and Kushner, S.R. (2000) Mol. Microbiol. 38, 854–866.
[14] Lopez, P.J., Marchand, I., Joyce, S.A. and Dreyfus, M. (1999) Mol. Microbiol. 33, 188–199.
[15] Li, Z. and Deutscher, M.P. (2002) RNA 8, 97–109.
[16] Ow, M.C. and Kushner, S.R. (2002) Genes Dev. 16, 1102–1115.
[17] Altman, S. (1989) Adv. Enzymol. 62, 1–36.
[18] Reed, R.E., Baer, M.F., Guerrier-Takada, C., Donis-Keller, H. and Altman, S. (1982) Cell 30, 627–636.
[19] Motamedi, H., Lee, Y. and Schmidt, F.J. (1984) Proc. Natl. Acad. Sci. USA 81, 3959–3963.
[20] Lee, Y., Ramamoorth, R., Park, C.-U. and Schmidt, F.J. (1989) J. Biol. Chem. 264, 5098–5103.
[21] Sakamoto, H., Kimura, N. and Shimura, Y. (1983) Proc. Natl. Acad. Sci. USA 80, 6187–6191.
[22] Gurevitz, M., Jain, S.K. and Apirion, D. (1983) Proc. Natl. Acad. Sci. USA 80, 4450–4454.
[23] Lundberg, U. and Altman, S. (1995) RNA 1, 327–334.
[24] Kim, S., Kim, H., Park, I. and Lee, Y. (1996) J. Biol. Chem. 271, 19330–19337.
[25] Reed, R.E. and Altman, S. (1983) Proc. Natl. Acad. Sci. USA 80, 5359–5363.
[26] Ehretsmann, C.P., Carpousis, A.J. and Krisch, H.M. (1992) Genes Dev. 6, 149–159.
[27] Sim, S., Kim, S. and Lee, Y. (2001) FEBS Lett. 505, 291–295.
[28] Kim, S., Sim, S. and Lee, Y. (1999) Nucleic Acids Res. 27, 895–901.
[29] Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene 33, 103–119.
[30] Bollag, D.M., Rozycki, M.D. and Edelstein, S.J. (1996) Protein Methods, 2nd edn., pp. 72–74, Wiley-Liss, New York.
[31] Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual, 3rd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[32] McDowall, K.J., Hernandez, R.G., Lin-Chao, S. and Cohen, S.N. (1993) J. Bacteriol. 175, 4245–4249.
[33] Li, Z., Pandit, S. and Deutscher, M.P. (1998) Proc. Natl. Acad. Sci. USA 95, 2856–2861.
[34] Yehudai-Resheff, S. and Schuster, G. (2000) Nucleic Acids Res. 28, 1139–1144.