In vitro analysis of processing at the 3′-end of precursors of M1 RNA, the catalytic subunit of Escherichia coli RNase P: multiple pathways and steps for the processing

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ABSTRACT

M1 RNA of 377 nucleotides, the catalytic subunit of Escherichia coli RNase P, is produced by a 3′ processing reaction from precursor M1 RNA, a major transcript from the rnpB gene. We analyzed products and intermediates generated by the in vitro processing reaction using a 40% ammonium sulfate precipitate of the S30 fraction (ASP-40) and determined their involvement in the processing. From the results we proposed a model of two pathways for 3′ processing of M1 RNA. In this model, one pathway (pathway I) involves +385/+386 intermediates and the other pathway (pathway II) does not. The position of the 3′-end of the precursor molecule determined the choice of the pathways. The precursor having the 3′-end of +413 was processed by both pathways while that having the +415 end was processed only by pathway II. The ASP-40 fraction generated processing products (termed +378/+379 RNA) containing one or two more nucleotides at the 3′-end than M1 RNA, regardless of which pathway was used. Therefore, both pathways require the final 3′-end trimming for complete processing. The endonucleolytic generation of +378/+379 RNA by pathway II was blocked by the mre-3071 mutation, suggesting that this step is carried out by RNase E.

INTRODUCTION

Ribonuclease P (RNase P) is a processing enzyme that catalyzes the endonucleolytic removal of 5′ leader sequences from precursors of tRNAs to generate the mature 5′-ends of tRNAs (1). M1 RNA of 377 nucleotides, which acts as the catalytic subunit of Escherichia coli RNase P (2), is formed by transcription from the rnpB gene (3–5) and subsequent processing of the resulting transcripts (6–10).

In vitro and in vivo studies indicate that the major primary transcript from the rnpB gene is the one initiating from the nearest promoter, P-1, and terminating at the first terminator, T1 (4,11,12). This primary transcript, termed precursor M1 RNA (pM1 RNA), carries an extra stretch of ~36 nucleotides containing a termination stem and loop at the 3′-end. These extra nucleotides are removed by a processing event in vivo. However, the processing event can hardly be described as a simple one. For instance, partially purified E.coli cell extracts generate larger products which have one or two more nucleotides at the 3′-end than the mature M1 RNA, implying multiple processing events in 3′ processing of M1 RNA (7,9). Although the processing activity has not been well characterized, several experiments (7–9) showed that it was related to RNase E activity (13). Although a recent study reported that the RNase E activity is correlated with the 3′ processing activity of M1 RNA (8), it was suggested that more enzymes should be required for complete processing of M1 RNA at the 3′-end (7,9). Indeed, larger products with up to six extra nucleotides at the 3′-end have been found in some E.coli strains defective in exoribonucleases (14), indicating that exoribonucleases are involved in the processing. However, it is unclear how these enzymes participate in the processing since the details of the processing reaction are not yet known. The RNase E endonuclease has been thought to be responsible for generating the larger product having one or two extra nucleotides at the 3′-end by endonucleolytic cleavage even though the downstream fragment expected directly from pM1 RNA by endonucleolytic cleavage has not been identified. Therefore, the in vivo findings of the larger products with up to six extra nucleotides at the 3′-end even puzzled us about 3′ processing pathways for maturation of M1 RNA. RNase E might cleave pM1 RNA to generate longer 3′-termini than expected and then RNase E-associated exoribonucleases would take over the next processing reaction for 3′ trimming. Another possibility is that the 3′-end of the upstream product formed by RNase E cleavage may be extended by a synthetic activity, such as poly(A) polymerase and then trimmed by exoribonucleases (15). Alternatively, the processing may occur by more than one pathway: one involving RNase E activity and the other not.

We previously showed that truncated pM1 RNAs derived from the internally deleted rnpB gene were processed to the corresponding truncated M1 RNAs in vivo (9). One of the truncated pM1 RNA transcripts, p23 RNA, has been used as a model substrate for determining features involved in 3′ processing of M1 RNA because it can be easily distinguished from endogenous M1 RNAs...
RNA (9). In this work, we investigated in vitro 3′ processing of M1 RNA in detail using p23 RNA in order to gain an insight into the 3′ processing pathways and the mechanism for maturation of M1 RNA. The results support a model that 3′ processing of M1 RNA occurs by two pathways. In this model, pM1 RNA is sequentially converted to the +385/+386 intermediates (having the 3′-end of +385 or +386), then to +378/+379 intermediates and, finally, the mature M1 RNA of 377 nucleotides. Alternatively, it is endonucleolytically cleaved to the +378/+379 intermediates, whose 3′-ends are then trimmed to form the mature 3′-end of +377.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids**

The *E. coli* RNase E+ and RNase Eis isogenic strains MCE+ (rne K12r-m+) and MCE– (rne-3071 K12r-m+) derived from MC1061 (16) were used for the preparation of cell extract. *Escherichia coli* JM109 (17) was used for the construction of plasmids as well as preparation of cell extract. Plasmid vectors used were pALTER-1 and pGEM-T from Promega. Plasmid pMTd23 (9) is a derivative of pGEM3, which contains the *rnpB* gene with an internal deletion of the sequence between +57 and +330. Plasmid pSPd23 (9) is a derivative of pALTER-1 containing the deleted *rnpB* gene of pMTd23, in which the SP6 promoter is linked to the 5′-end of the M1 RNA coding sequence and a DraI site at position +415 is generated for in vitro run-off transcription. Plasmid pLM1 (18) is a derivative of pGEM3 containing the intact *rnpB* gene. The plasmid used for generating antisense RNA complementary to the 3′-terminal region of pM1 RNA was pGEM3(±369) carrying the *rnpB* fragment between +369 and +467 (S.Sim and Y.Lee, unpublished results).

**Enzymes**

SP6 and T7 RNA polymerase, T4 polynucleotide kinase and restriction enzymes were purchased from Promega. Plasmid vectors as well as preparation of cell extract. Plasmid vectors used were pALTER-1 and pGEM-T from Promega. Plasmid pMTd23 (9) is a derivative of pGEM3 containing the *rnpB* gene (9). *Escherichia coli* RNA polymerase (1.5 pmol) was incubated at 37°C for 5 min in 16 µl reaction buffer [30 mM Tris–HCl, pH 8.0, 3 mM MgCl2, 0.1 mM EDTA, 0.2 mM dithiothreitol (DTT), 100 mM KCl] with 0.15 pmol of template DNA. Single round transcriptions were started by the addition of 3.5 µl of a nucleotide mixture containing 0.4 µM [α-32P]CTP and 0.4 mM each of ATP, UTP and GTP. After 2 min, 3 µl of 1 mg/ml heparin was added and the incubation was continued for 5 min before adding 2.5 µl of 12 mM cold chase CTP. Reactions were terminated by phenol extraction 10 min after the CTP addition and RNA was precipitated in ethanol.

**In vitro transcription**

For in vitro transcription of RNA substrates to be used for the in vitro processing assay, plasmid DNAs were cleaved with appropriate restriction enzymes such as *DraI*, *Smal*, *FokI* or *RsaI* and used as templates for run-off transcription by SP6 RNA polymerase. In vitro transcription products were purified by gel elution as described (19).

**In vitro processing assay**

The 40% ammonium sulfate precipitation (ASP-40) was prepared from *E. coli* JM109 and rne+ and rne-3071 isogenic strains as described (9). In the case of *rne* isogenic strains, cells were grown at 30°C to an *A*600 of ~0.6 and then shifted to 44°C for 1 h. Cells were harvested before and after the temperature shift. The in vitro processing assay was performed in 30 mM Tris–HCl, pH 7.5, 60 mM NH4Cl, 10 mM MgCl2, 0.5 mM DTT, 0.1 mM phenylmethylsulfonylfluoride, and 2% polyethylene glycol as described (9). Yeast total RNA of 170 µg/ml was added into the reaction mixture when required. In the case of the in vitro assay using the isogenic strains, RNA substrates were incubated at 30 or 44°C with ASP-40 from the cells grown at the respective temperatures. For the assays at 44°C, ASP-40 was preincubated at 44°C for 30 min before it was added into the reaction mixtures. The reaction products were extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and RNA was analyzed by northern hybridization or electrophoresis on a polyacrylamide sequencing gel containing 8 M urea.
were intramolecularly ligated in 30 mM HEPES, pH 7.5, 20 mM MgCl₂, 3 mM DTT, 0.1 mM ATP, and 10% v/v dimethyl sulfoxide, 10% v/v dimerethyl sulfoxide, 0.1 mM ATP, 10% v/v dimethyl sulfoxide, 10 µg/ml BSA) with 11 U of RNA ligase at position +415 and then labeled with 32P γ-CTP and +386 RNA was internally labeled with [α-32P]CTP. Substrate RNAs were incubated at 37°C for 5 min with partially purified cell extract (ASP-40) from JM109 and analyzed on a 5% polyacrylamide sequencing gel containing 7 M urea. The substrate RNAs used are shown above each lane. +385/+386 RNA gel purified from the reaction products of p23 RNA is indicated as intermediate. The presence and absence of ASP-40 or excess yeast total RNA (yT RNA) are indicated as plus (+) and minus (−), respectively. G and A stand for G-specific (RNase T1) and A-specific (RNase U2) cleavage products of p23 RNA, respectively. The nucleotide positions of the rnpB gene are indicated on the left side of the figure.

**RESULTS**

**Analysis of upstream products or intermediates generated by in vitro 3’ processing reaction**

A truncated pM1 RNA, p23 RNA (Fig. 1), which is derived from the internally deleted rnpB gene, has been successfully used as a model substrate for studying 3’ processing of M1 RNA in vivo because the intact M1 RNA sequence is not necessary for the processing reaction (9). p23 RNA has a deletion of the sequence between +57 and +330 of M1 RNA and an insertion of eight nucleotides as the EcoRI linker. To elucidate the mechanism in 3’ processing of M1 RNA for generating the mature 3’-end of +377, first, we analyzed in vitro processing reaction products generated from p23 RNA by a partially purified cell extract that was prepared as a 40% ammonium sulfate precipitate of S30 fraction (ASP-40). p23 RNA was obtained by in vitro transcription from the pSPd23 DNA cleaved with Drai at position +415 and then labeled with 32P at the 5’-end. The labeled RNA was assayed with ASP-40 to characterize reaction products. In some in vitro reactions, we included excess yeast total RNA with the hope that it might inhibit specific steps in the processing reaction. This excess yeast total RNA was previously added to the reaction mixture to detect metabolically unstable reaction products, caused by disruption of a base pairing region, because they were rapidly degraded by unknown nuclease activities of ASP-40 just after processing and these activities were inhibited by excess yeast total RNA (9). ASP-40 generated upstream RNA products with the 3’-ends at +378 and +379 in the absence of excess yeast total RNA. However, the presence of excess yeast total RNA with the oligonucleotide-specific primer and FOR primer. From both methods, the DNA fragments amplified by PCR were cloned into the pGEM-T vector and sequenced.
RNA resulted in additional products (termed +385/+386 RNA) with the 3′-ends at +385 and +386 (Fig. 2). Control experiments showing that yeast total RNA alone had no cleavage activity in the reaction condition without ASP-40 (data not shown) excluded the possible involvement of yeast total RNA in generating these +385/+386 RNA. Therefore +385/+386 RNA might result from inhibition of their further processing by excess yeast total RNA, implying that they are involved in the 3′ processing of M1 RNA. Since the +385/+386 RNA could be the metabolites simply in the degradation pathway that could not give rise to the processed products, we tested whether +385/+386 RNA was further processed or not. The +385/+386 RNA band was eluted from the gel or +386 RNA was synthesized by in vitro transcription. For constructing the DNA template for in vitro transcription, an Rsal site was introduced at +386. Both the RNA eluted from the gel and the in vitro transcript derived from the Rsal-digested DNA template were processed to generate +378/+379 RNA products (whose 3′-ends were at +378 and +379) and appeared not to go into the degradation pathway (Fig. 2). In addition, they were not processed in the presence of excess yeast total RNA as expected. These results suggest that +385/+386 RNA participates as intermediates in the processing reaction. In this in vitro processing reaction, the +386 RNA substrate also generated products with more 3′ residues than +378/+379 RNA having one or two extra 3′ residues. This might arise from partial loss of processivity by a disruption of possible cooperation of enzymes participating in the processing. ASP-40 converted p23 RNA not only into +385/+386 RNA but also into +378/+379 RNA in the presence of excess yeast total RNA that inhibited further processing of +385/+386 RNA into +378/+379 RNA (Fig. 2). Therefore the processing of p23 RNA should occur by another pathway which does not involve the +385/+386 RNA intermediates.

**Presence of multiple pathways for 3′ processing of M1 RNA**

We tried to determine the identities of downstream products that could be generated if the upstream products, such as +378/+379 or +385/+386 RNA, were formed by endonucleolytic cleavage at a single site. Since 3′-ends of an in vitro transcribed RNA are usually heterogeneous, in vitro transcribed p23 RNA was resolved on a long gel. Two major bands were eluted from the gel and their 3′-ends were determined by 5′–3′ ligation and sequencing (data not shown): one has a 3′-end of +415 and the other +413. Therefore, the terms p23 RNA (+415) and p23 RNA (+413) were used to denote the substrates for the following processing reactions. We initially tested each substrate in vitro for the processing. Northern hybridization of the reaction products surprisingly indicated that the processing event of p23 RNA (+413) involved the +385/+386 RNA intermediates, but that of p23 RNA (+415) did not (Fig. 3). When the 3′-end-labeled p23 RNA (+415) with [32P]pCp was used, downstream products of 38 and 37 nucleotides were detected, which correspond to those endonucleolytically cleaved after positions +378 and +379, respectively (Fig. 4A). p23 RNA (+413) labeled with [32P]pCp also yielded the corresponding 3′ fragments (36 and 35 nucleotides including pCp). One may argue that processing with substrates tagged with pCp at the 3′-end might differ from that with untagged substrates. In vitro assays with internally labeled substrates (Fig. 4B) or northern analysis of products with an antisense RNA of the 3′-terminal region as a probe (Fig. 4C) showed the same results, confirming that both p23 RNA (+413) and p23 RNA (+415) were able to generate +378/+379 RNA by endonucleolytic cleavage. Taken together, therefore, it can be proposed as a model of processing pathways for 3′ maturation of M1 RNA that p23 RNA (+413) is processed to +378/+379 RNA both by a two-step process (termed pathway I) involving the +385/+386 intermediates and by a one-step process (termed pathway II), while p23 RNA (+415) is processed solely by pathway II. We also observed products of ~26–27 nucleotides by northern analysis with the antisense RNA of the 3′-terminal region as a probe (Fig. 4C). The band of ~26–27 nucleotides was detected from p23 RNA (+413) in the absence of excess yeast total RNA, suggesting that +385/+386 RNA intermediates might be produced along with the downstream products of 27 and 26 nucleotides by endonucleolytic cleavage. In the presence of excess yeast total RNA, we were unable to detect the band due to high background signals which resulted from cross-hybridization of excess yeast total RNA with the probe. When the intact precursor M1 RNAs were incubated with ASP-40, the corresponding +385/+386 RNA intermediates were generated only from pM1 RNA (+413) in the presence of excess yeast total RNA, while the corresponding +378/+379 RNAs were formed from both pM1 RNA (+415) and pM1 RNA (+413) (Fig. 5). This result indicates that the multiple pathways in 3′ processing of M1 RNA were not an artifact resulting from the use of the artificial substrates containing the internal deletion of the M1 RNA sequence.

In order to determine the natural termination site of the rnpB gene, in vitro transcription with E.coli RNA polymerase was performed using pMTd23 (9) as a template, which contains the same internally deleted rnpB sequences with its own promoter and terminator (Fig. 6). Escherichia coli RNA polymerase produced transcripts terminating at positions +413 (major) and +415 (minor), indicating that the 3′-ends of p23 RNA (+415) and p23 RNA (+413) were equivalent to those of natural primary transcripts of the rnpB gene.

![Figure 3. Time course of in vitro processing of p23 RNA (+415) and p23 RNA (+413)](image-url)
Figure 4. Identification of downstream reaction products. RNA substrates labeled at the 3′-end with [32P]pCp (A) and internally labeled with [α-32P]CTP (B) were incubated with ASP-40 and analyzed on 10% polyacrylamide sequencing gels. For generation of size markers, p23 RNA (+415) labeled at the 3′-end was used. OH indicates alkaline hydrolysis ladders. G and A stand for G-specific (RNase T1) and A-specific (RNase U2) cleavage products, respectively. p23 RNA (+413) labeled at the 3′-end is referred to as +413/+412 because it was contaminated with p23 RNA (+412) during gel purification. Reaction times are shown above each lane. (C) Unlabeled RNA substrates were incubated with ASP-40 for 30 min and the reaction products were analyzed by northern hybridization as in Figure 3 except that RNAs were electrophoresed on an 8% polyacrylamide gel. The probe was an antisense RNA to the 3′-terminal region (from positions +367 to +417) of pM1 RNA. The nucleotide sizes, which are estimated from the size markers, are indicated on either side of the figures.

Figure 5. In vitro 3′ processing of intact precursor M1 RNA (pM1 RNA). RNA substrates were synthesized by in vitro transcription. The transcription products were resolved on a 5% polyacrylamide gel containing 7 M urea and pM1 RNA (+415) and pM1 RNA (+413) were purified by gel elution. The purified RNAs were incubated with ASP-40 from JM109 at 37°C and subjected to northern analysis as in Figure 3.

Figure 6. In vitro transcripts of the rnpB gene by E.coli RNA polymerase. In vitro transcription with E.coli RNA polymerase was performed using pMTd23 as the template, which contains the internally deleted rnpB (9). The control reaction was performed using pGEM3 as the template. The resulting RNA products were analyzed in a sequencing gel. The template DNAs used are shown above each lane. As size markers, internally labeled p23 RNA (+415) and p23 RNA (+413) were used, which are indicated as +415 and +413, respectively.

The processing pathways are determined by the length of the 3′-end, not by its sequence

In an effort to investigate sequence requirements for pathway I, the UUU sequence at positions +413 to +415 was changed to UCG, UCC, UGG, UUC or CCC (underlined sequences denote the changes; Fig. 1). The +415 variants were processed by the one-step process without +385/+386 RNA intermediates as was the wild-type p23 RNA (+415) (Fig. 7), suggesting that the on/off choice of pathway I depends on the length of the 3′-end rather than its sequence.

rne dependency of pathways I and II

Since the 3′ processing activity of M1 RNA is related to RNase E (7–9), rne dependency of each pathway was tested using E.coli strain MCE– (16) carrying the rne-3071 mutation responsible for thermosensitivity of RNase E function. p23 RNA (+413) and p23
RNA (+415) were used for in vitro processing reactions at 30 or 44°C with ASP-40 from isogenic MCE+ (rne+ or MCE- (rne-3071) strains. The reaction products were then analyzed by northern hybridization. The +378/+379 RNA products were not detected with ASP-40 from JM109 except at 44°C as expected (Fig. 8). In contrast, these products were not detected with ASP-40 from the rne+ strain at either temperature or the rne5 strain at 30°C (Fig. 8). These results indicate that processing by pathway II is rne-dependent because p23 RNA (+415) is processed only by pathway II. On the other hand, the conversion of p23 RNA (+415) to the +385/+386 RNA intermediates by pathway I was a little complex. When p23 RNA (+413) was incubated with ASP-40 from the wild-type or mutant strains, the intermediates were detected in the reaction at 30°C as expected (Fig. 8). However, the reaction at 44°C did not generate the intermediates regardless of the source of cell extracts. When ASP-40 prepared from a different strain, JM109, was used, the intermediates were still observed at 44°C even though the amount of the intermediates decreased with an increase in reaction temperature (Fig. 9). In contrast, the +386 intermediate RNA was further processed into +378/+379 RNA by ASP-40 from both rne+ and rne-3071 isogenic strains at either temperature (Fig. 8), indicating that this step in pathway I was not affected by the rne-3071 mutation.

DISCUSSION

In this study, we analyzed in vitro products and intermediates in the in vitro 3' processing reaction of M1 RNA with ASP-40 as the partially purified cell extract and determined their involvement in the reaction pathways. From these in vitro results we propose a model of 3' processing pathways for maturation of M1 RNA. This model consists of two different pathways (Fig. 10). In pathway I, precursor RNA molecules are cleaved to generate the +385/+386 RNA intermediates, which are subsequently processed to +378/+379 RNA by another processing activity. In pathway II, RNAs are directly processed to generate +378/+379 RNA. Since the final processing products of the in vitro reaction are +378/+379 RNA regardless of the pathways, 3' trimming reaction of one or two extra nucleotides must occur to generate the mature 3'-end of +377. We also show that p23 RNA (+415) (having the 3'-end of +415) is processed only by pathway II, but p23 RNA (+413) (having the 3'-end of +413) is processed by both pathways I and II. Since in vitro transcripts terminating at the T1 terminator of rnpB (10,12) by E.coli RNA polymerase contain both ends of +413 and +415, the ends of p23 RNA (+413) and p23 RNA (+415) used as substrates in this study appear to be identical to those of natural primary transcripts of rnpB.

The pathway by which 3' processing of M1 RNA occurs in vivo is still unknown. Although the in vivo processing appears to require RNase E and exoribonucleases, it has not been clear how these enzymes are involved in the processing. It has been suggested that RNase E is responsible for generating RNA products having one or two more nucleotides (termed +378/+379 M1 RNA) from pM1 RNA (7–9,24). On the other hand, exoribonucleases might play important roles in 3' maturation of M1 RNA because molecules with up to six extra 3' residues accumulated in an E.coli strain lacking multiple exoribonucleases (14). Since +378/+379 M1 RNA (having one or two more nucleotides at the 3'-end) can be formed directly from pM1 RNA by single endonucleolytic cleavage as shown in this study for the first time (Fig. 4), the in vivo presence of processing intermediates with as many as six extra 3' nucleotides suggests that in vivo 3' processing of M1 RNA also seems to occur by more than one processing pathway. Therefore, our model with two different pathways explains well the in vivo situation for 3' processing of M1 RNA, implying that both pathways are also functional in vivo.

Both p23 RNA (+413) and p23 RNA (+415) are processed by pathway II. In contrast, only p23 RNA (+413) can be processed...
dependent (Fig. 8), different processing of p23 RNA (+413) and formation of the processing intermediates was temperature demonstrated, but may be a sequence-specific endoribonuclease step of pathway I generating +385/+386 RNA remains to be cleavage by RNase E. An enzyme activity responsible for the first step of pathway II occurs with a single endonucleolytic downstream products, our present study clearly shows that the high temperatures (Fig. 8). With the identification of the absence of two nucleotides at the 3′-end although they were less than at 30°C, C, while they were observed with ASP-40 from strain JM109. Moreover, Li et al. (14) have argued that 3′-exoribonucleolytic trimming is required for the final maturation of M1 RNA by showing that large amounts of immature molecules with up to six nucleotides at the 3′-end accumulated in E.coli cells when multiple exoribonucleases were absent. The first step of pathway II was not inhibited by excess yeast total RNA, suggesting that RNase E activity is not affected by excess yeast total RNA. In contrast, the second step of pathway I was blocked by this excess RNA. It is noteworthy, therefore, that the +385/+386 RNA intermediates did not act as substrates for RNase E even though they had the rne-dependent site. This suggests that the downstream sequences from +386 are required for RNase E to recognize pM1 RNA. However, it remains to be determined why the second step of pathway I stops at +378 and +379 in the in vitro processing reaction with ASP-40. Finally, one or two nucleotides at the 3′-end of +378/+379 RNA intermediates should be trimmed by a processing activity which might be lost in the ASP-40 preparation.

In summary, we have shown evidence that 3′-processing of M1 RNA occurs by two pathways. One pathway (pathway I) involves the +385/386 intermediates and the other (pathway II) does not. We have also mapped the acute cleavage sites of the first step of pathway II by observing the upstream and downstream cleavage products which appear to result from cleavage by RNase E activity. The length of 3′-ends of precursor molecules determines by which pathway(s) the processing occurs. The processing does not occur through a simple step but involves complex and multiple steps. Therefore, many enzymes are involved in 3′-processing of M1 RNA, and even in pathway II at least two enzyme activities are required for complete processing. It is not clear yet, however, why 3′-processing of M1 RNA occurs by two pathways. The answer to this question will enhance our understanding of the importance of M1 RNA processing in vivo, particularly in the biosynthesis of RNase P. The synthesis rate of RNase P is expected to vary with growth conditions, as does the translation apparatus such as ribosomes and tRNA (26,27). The M1 RNA processing may play an important role in growth-dependent regulation for the synthesis of RNase P because there is some evidence that RNase processing or degradation activities, including RNase E, are also regulated by growth conditions (28–30).

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**REFERENCES**


**Figure 10.** A proposed model of 3′ processing pathways of M1 RNA. Only the stem–loop structure downstream of the processing site is represented as a secondary structure. Pathway I involves the +385/+386 RNA intermediates and pathway II does not. The final trimming step of one or two nucleotides from +378/+379 RNA to generate the mature M1 RNA with the 3′-end of +377 is also represented.