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Rapid degradation of polyadenylated *oop* RNA

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Abstract The *oop* RNA is a short (77 nucleotides (nt)) transcript encoded by bacteriophage λ which acts as an antisense RNA for λ *cII* gene expression. Recently we demonstrated that *oop* RNA is specifically polyadenylated at its 3′ end by poly(A) polymerase I (PAP I), the *pcnB* gene product. Here we demonstrate that the half life of *oop* RNA is 3 times longer in the *pcnB* mutant relative to the *pcnB*$^+$ host, indicating that polyadenylation of this transcript causes its accelerated degradation. Although it was proposed that polyadenylation of RNAs in bacteria leads to their enhanced degradation, in most cases stabilization of these molecules was observed only when other mutations (*pnp*, *rnb* and *rne*) were present in the *pcnB*$^-$ strain. Therefore it seems that *oop* RNA may serve as a very useful model in further studies on molecular mechanisms of RNA polyadenylation and degradation in bacteria. Analysis of *oop* RNA and its degradation product isolated from *Escherichia coli* cells suggests that both polyadenylated and non-modified *oop* transcripts can act as antisense RNA.

Key words: RNA polyadenylation; *pcnB* gene (*Escherichia coli*); RNA degradation; *oop* RNA (bacteriophage λ)

1. Introduction

Despite the fact that poly(A) RNA was discovered in bacteria over twenty years ago, studies on the role of polyadenylation at the 3′-end of mRNA in prokaryotic cells were significantly less extensive relative to those performed with eukaryotic cells [1]. Nevertheless, it seems that RNA polyadenylation may be a common and very important process in prokaryotes. It was demonstrated that mRNA, but not stable RNAs, can be a target for polyadenylation in bacteria [2]. It is generally accepted that polyadenylation leads to destabilization of RNA in prokaryotic cells, however, in most cases less efficient degradation of RNA molecules in strains defective in polyadenylation was observed only when additional mutations in genes coding for enzymes involved in RNA decay (*pnp*, *rnb* and *rne*) were present in hosts defective in polyadenylation [1].

There are two enzymes capable of RNA polyadenylation in *Escherichia coli*: poly(A) polymerase I (PAP I), the *pcnB* gene product [3], and PAP II whose gene has been identified recently [4]. It seems that simultaneous lack of these two enzymes is lethal for *E. coli* [4].

Recently we found that a short transcript encoded by bacteriophage λ, the *oop* RNA, is specifically polyadenylated at its 3′-end by PAP I [5]. The *oop* RNA is an antisense transcript for expression of the λ *cII* gene, whose product is an activator of promoters indispensable for lysogenic development of λ. We demonstrated that lysogenization of *pcnB* mutants by wild-type λ phage, but not by a mutant phage unable to produce *oop* RNA, is impaired [5]. The *oop* RNA was also shown to be more abundant in the *pcnB* mutant relative to wild-type bacteria [5]. These results suggested that polyadenylation of *oop* RNA may cause enhanced degradation of this transcript. Because of the strong phenotypic effect of *pcnB* mutations on phage λ development, it was possible that stabilization of *oop* RNA could be observed in cells devoid of PAP I even in the absence of additional mutations in genes coding for ribonucleases. The aim of this work was to test this hypothesis.

2. Materials and methods

Escherichia coli wild-type (MG1655) strain and its *pcnB80* derivative [5] were used. The pBW6 plasmid [5] contains the pSC101 *ori*, wild-type *oop* locus and a fragment of the p_R operon (with *cro*, *cII* and truncated *O* genes). Abundance of the *oop* RNA in *E. coli* cells bearing pBW6 was estimated by S1 nuclease protection assay (using S1 Nuclease Protection Kit, Ambion) as described previously [5].

3. Results

The analysis of the *oop* RNA isolated from *E. coli* cells bearing plasmid pBW6 revealed two bands interacting with a probe specific to *oop* RNA [5]. These two bands were observed in both *pcnB*$^+$ and *pcnB80* hosts [5]. One of these bands was of expected length for *oop* RNA (77 nt), and the second band migrated somewhat faster (Fig. 1). We suspected that the shorter RNA is a product of *oop* RNA degradation. To calculate precisely the length of the shorter RNA, we repeated the detection of *oop* RNA but using conditions which allowed us to obtain a ladder of bands following S1 nuclease digestion (a proper concentration of S1 nuclease and digestion time was chosen according to instructions of the manufacturer (Ambion) of the S1 Nuclease Protection Kit, and a long sequencing gel was used). We found that the smaller RNA was several nucleotides shorter than *oop* RNA (Fig. 1). It was demonstrated previously that the *oop* RNA-*cII* mRNA hybrid is cut by RNase III 13 nucleotides from the 5′-end of *oop* RNA [6,7]. Therefore, most probably the shorter RNA observed in Fig. 1 is a product of the cleavage of *oop* RNA by RNase III.

In order to estimate half life of *oop* RNA in *pcnB*$^+$ and *pcnB80* hosts, bacteria were treated with rifampicin (200 μg/ml) and samples for RNA isolation were withdrawn before treatment and at indicated times after rifampicin addition. We found that degradation of *oop* RNA was significantly quicker in the *pcnB*$^+$ strain relative to the *pcnB80* mutant (Fig. 2). Similar results were obtained for the shortened transcript (Fig. 3). We calculated that half life of *oop* RNA (and its

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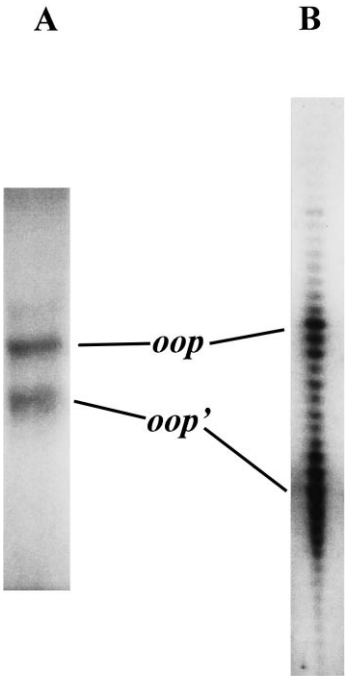

Fig. 1. Detection of *oop* RNA (*oop*) and its shortened derivative (*oop′*) in the S1 nuclease protection assay (using S1 Nuclease Protection Kit (Ambion) and according to a procedure described in [5]). In the experiment presented in panel A, standard conditions, described earlier [5], were used. In the experiment presented in panel B, conditions allowing to obtain a ladder of bands following S1 nuclease digestion were used (a proper concentration of S1 nuclease and digestion time was chosen according to the instructions of the manufacturer of the S1 Nuclease Protection Kit (Ambion), and RNAs were separated on a long sequencing gel).

shorter derivative) is 3 times longer in the *pcnB80* host than in the wild-type bacteria (Table 1).

4. Discussion

Although for many years polyadenylation of the 3′-end of RNA was investigated mainly in eukaryotic cells, recent reports indicated that this process occurs also frequently in bacteria and that it seems to be of great importance for the regulation of gene expression [1]. In fact, there are more and more communications indicating that many bacterial RNAs are polyadenylated [8–10]. We found recently that *oop* RNA (encoded by bacteriophage λ) is also polyadenylated at its 3′-end.

It was proposed that RNA polyadenylation in bacterial cells results in its accelerated degradation. Here we demonstrate that in the mutant defective in the PAP I function (*pcnB80*) half life of *oop* RNA is significantly longer than in wild-type bacteria. This finding is in accordance to the hypothesis presented above. Moreover, *oop* RNA, which is ordinarily polyadenylated in the wild-type host, is one of very few examples of decreased rate of degradation of polyadenylated form of RNA in a *pcnB*$^-$ host in the absence of additional mutation(s) in gene(s) coding for protein(s) involved in RNA turnover [1]. The *oop* RNA is a very short transcript (77 nt) forming a relatively simple secondary structure [5]. This transcript is produced by bacteriophage λ but it is dispensable for *E. coli* growth. Therefore, we suspect that *oop* RNA may be a very useful model in further studies on mechanisms of RNA polyadenylation and degradation in bacteria.

Table 1
Half life of *oop* RNA and its shortened form (*oop′* RNA) in *Escherichia coli pcnB*$^+$ and *pcnB80* hosts

Host	Half life (min)	
	oop RNA	*oop′* RNA
pcnB$^+$	1.4	1.5
pcnB80	4.3	4.6

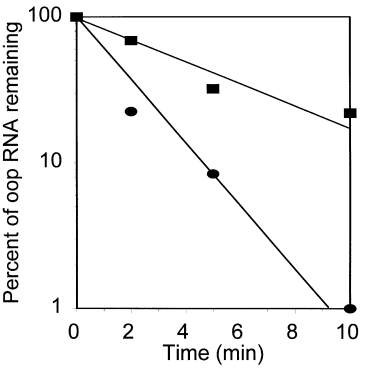

Fig. 2. Stability of *oop* RNA in *pcnB*$^+$ (circles) and *pcnB80* (squares) hosts. Total RNA was isolated from cells bearing plasmid pBW6 before (time 0) and at indicated times after treatment with rifampicin (200 μg/ml), and *oop* RNA was detected as described earlier [5] and as presented in Fig. 1A. The bands on an autoradiogram were quantitated by densitometry.

We demonstrated previously that *oop* RNA plays a role in the 'lysis-versus-lysogenization' decision of bacteriophage λ [5]. This transcript is an antisense RNA for *cII* gene expression as the *oop* RNA-*cII* mRNA hybrid is cleaved by RNase III at a specific site 13 nucleotides from the 5′-end of *oop* RNA [6,7]. Our results indicate that this cleavage occurs in both *pcnB*$^+$ and *pcnB80* strains, strongly suggesting that both polyadenylated and non-polyadenylated forms of *oop* RNA can form a hybrid with *cII* mRNA. Formation of such a hybrid results in negative regulation of *cII* gene expression as RNase III cleavage provokes further degradation of *cII* mRNA [6]. Therefore, *oop* RNA can act as an antisense transcript irrespective of its polyadenylation. This is compatible with our statement that polyadenylation of *oop* RNA reduces

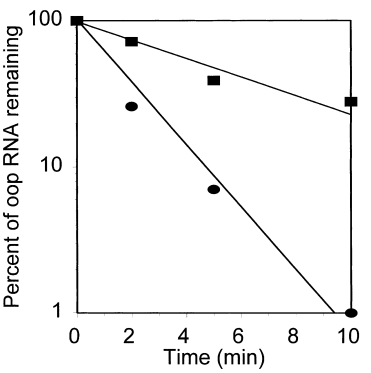

Fig. 3. Stability of the shortened derivative of *oop* RNA (*oop′*) in *pcnB*$^+$ (circles) and *pcnB80* (squares) hosts. Total RNA was isolated from cells bearing plasmid pBW6 before (time 0) and at indicated times after treatment with rifampicin (200 μg/ml), and *oop* RNA was detected as described earlier [5] and as presented in Fig. 1A. The bands on an autoradiogram were quantitated by densitometry.

half life of this transcript and may play an important role in the regulation of phage λ development.

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