Plasmid 62 (2009) 83–87

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

Arginine-rich RNA binding domain and protein scaffold domain of RNase E are important for degradation of RNAI but not for that of the Rep mRNA of the ColE2 plasmid

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ARTICLE INFO

Article history:
Received 7 January 2009
Revised 27 April 2009
Available online 6 May 2009

Communicated by Richard Calendar

Keywords:
Antisense RNA
Degradosome
PNPase
RhlB
RNase E
RNA degradation

ABSTRACT

Expression of the replication initiator protein (Rep) of the ColE2 plasmid is controlled by antisense RNA (RNAI). Therefore alterations in processes and/or rates of degradation of these two RNAs would affect the Rep expression. Here, we have shown that the arginine-rich RNA binding domain (ARRBD) of RNase E is important for the initial endoribonucleolytic cleavage of RNAI but dispensable for the endoribonucleolytic cleavages of the Rep mRNA. We have also shown that the protein scaffold domain of RNase E is important for successive exoribonucleolytic degradation of RNAI, suggesting involvement of RhlB, but dispensable for that of the Rep mRNA. Such differences in the initiation and successive steps of degradation between RNAI and the Rep mRNA might be important in determining their individual degradation efficiencies required for a quick response to the changes in the plasmid copy number.

1. Introduction

Stability of mRNA is one of the parameters for determining the efficiency of gene expression. Mechanisms of mRNA decay in bacteria have been extensively studied (Kushner, 2002; Arraiano and Maquat, 2003; Deutscher, 2006). RNase E is the major endoribonuclease in RNA degradation and processing in *Escherichia coli* (Cohen and McDowall, 1997). The N-terminal half of RNase E is the catalytic domain for the endoribonuclease activity and in the C-terminal half there are the arginine-rich RNA binding domain (ARRBD) and protein scaffold domain for formation of the RNA degradation complex (degradosome) (McDowall and Cohen, 1996). The ARRBD is important for the RNase E processing of 9S rRNA (Kaberdin et al., 2000) and it has been proposed that the ARRBD might be important for RNase E binding to highly structured RNA substrates. The degradosome contains RNase E, PNPase, RhlB, enolase, and other factors (Py et al., 1994, 1996; Miczak et al., 1996; Vanzo et al., 1998). The scheme of the degradosome action is as follows: PNPase promotes rapid RNA degradation in the 3′–5′ direction after the RNase E cleavage, and RhlB unwinds RNA secondary structures and helps PNPase to accomplish rapid RNA degradation. The complex formation contributes to coordination of the endoribonucleolytic cleavage and successive exoribonucleolytic degradation (Py et al., 1994, 1996; Miczak et al., 1996; Vanzo et al., 1998).

The replication of the ColE2 plasmid DNA is initiated by the plasmid-coded replication initiator protein (Rep)[1] (Horii and Itoh, 1988; Itoh and Horii, 1989). The Rep expression is kept constant through inhibition of the translation of the Rep mRNA by antisense RNA (RNAI) (Takechi et al., 1994; Yasueda et al., 1994). We have previously shown that the plasmid copy number in the Δ*pcnB* mutant strain decreases to about a half of that in the wild-type (Nishio and

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[1] *Abbreviations used:* Rep, replication initiator protein; ARRBD, arginine-rich RNA binding domain.

0147-619X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.plasmid.2009.04.002

Itoh, 2008b). We have also shown that the RNAI degradation intermediate (RNAI*) accumulates in the Δ*pcnB* mutant strain. Based on the results of the RNAI degradation analysis, we have proposed a model for the RNAI degradation pathway. RNAI degradation is initiated by the RNase E cleavage at multiple positions about 10 bases from the 5′ end of RNAI, resulting in formation of RNAI degradation intermediate (RNAI*). PAP I adds a poly(A) tail to the 3′ ends of RNAI* and finally RNase II and PNPase degrade the poly(A)-tailed RNAI* from the 3′ end. On the other hand the Rep mRNA is degraded through complex degradation pathways (Nishio and Itoh, 2008a). In the Rep mRNA degradation, RNase E and RNase II play major roles and other ribonucleases are also involved in the degradation processes. Such different degradation processes of RNAI and the Rep mRNA might ensure their individual turn-over rates required for the efficient antisense RNA regulation.

Here, we analyzed RNAI and the Rep mRNA degradation processes in various *rne* deletion mutant strains, which lack the protein scaffold domain and the ARRBD. We have found that the ARRBD is important for the initial endoribonucleolytic cleavage of RNAI, but dispensable for the endoribonucleolytic cleavage of the Rep mRNA. We have also shown that the protein scaffold domain of RNase E is important for successive exoribonucleolytic degradation of RNAI, suggesting involvement of RhlB, but again dispensable for that of the Rep mRNA. Such differences in the degradation processes between RNAI and the Rep mRNA might be the cause of the different degradation efficiencies.

2. Materials and methods

2.1. Bacterial strains and plasmids

The *E. coli* K-12 strains and plasmids used have been described elsewhere (Horii and Itoh, 1988; Rao and Rogers, 1997; Nishio and Itoh, 2008a,b) except for those described below or listed in Table 1. The strains in Table 1 are kind gifts from Dr. S.R. Kushner.

2.2. Construction of plasmids

To construct pBAD24repKm, the 2.4-kb EcoRI–BamHI fragment (flush-ended) of pKC7 (Rao and Rogers, 1997) containing the kanamysin resistant gene was inserted into the ClaI site (flush-ended) of pBAD24rep (Nishio and Itoh, 2008a). To construct pBAD24repCm, the 2.4-kb BamHI fragment (flush-ended) of pEC22 (Horii and Itoh, 1988) containing the chloramphenicol resistance gene was inserted into the ClaI site (flush-ended) of pBAD24rep. To construct pEK22, the 2.4 kb EcoRI–BamHI fragment (flush-ended) of pKC7 containing the kanamysin resistance gene was ligated with the 2.4-kb BamHI fragment (flush-ended) of pEC22 containing the ColE2 plasmid replicon. The details of the construction of these plasmids are illustrated in the Supplemental Fig. 1.

2.3. Preparation of RNA

For RNA analysis, we used the mutant strains with the same genetic background listed in Table 1. Preparation of total RNA was performed mainly as described (Söderbom et al., 1997; Nishio and Itoh, 2008a,b), except that we changed the growth temperatures. Bacterial strains harboring pEK22 or pEC22 (for the RNAI analysis) and pBAD24repKm or pBAD24repCm (for the Rep mRNA analysis) were grown at 30 °C, until they reached to the mid log phase and then incubated at 37 °C for 10 min for the *rne* deletion mutant strains and wild-type strain or at 44 °C for 10 min for the *rne*-1 mutant strain. For the RNAI analysis, total RNAs were extracted at this moment. For the Rep mRNA analysis 0.2% arabinose was added after temperature shift and incubation was continued for 10 min at 37 °C for the *rne* deletion mutant strains and wild-type strain or at 44 °C for the *rne*-1 mutant strain.

2.4. Northern blot analysis

The Northern blot analyses of RNAI and the Rep mRNA were performed as described (Nishio and Itoh, 2008a,b) by using total RNA prepared above. To detect RNAI, the probe was prepared by using SP6 RNA polymerase (Promega) and DIG RNA labeling kit (Roche) with the PCR product, as template for transcription, which was amplified with the M13 universal primers and pGEMT-Str1-4 (Nishio and Itoh, 2008a,b) as template. Hybridization was performed at 68 °C for 12 h in DIG easy Hyb buffer (Roche). Detection was performed with the NBT/BCIP detection reagent (Roche). To detect the Rep mRNA, the probe was prepared by using T7 RNA polymerase (Promega) and DIG RNA labeling kit (Roche) with the PCR product, as template for transcription, which was amplified with the M13 universal primers and pGEMT-Str1-4 (Nishio and Itoh, 2008a,b) as template. Hybridization and detection were performed as above.

3. Results and discussion

3.1. Effects of the rne deletion mutations on the degradation process of ColE2 RNAI

To examine possible involvement of degradosome formation in the RNAI degradation process, we used three

Table 1
Bacterial Strains used in this study.

Strain	Bacterial genotype/plasmid genotype	Reference
SK9714	rneA1018::bla thyA715 rph-1 recA56 srl-300::TnlO Tc^r/pSBKl (rne + Cm^1)	Ow et al. (2000)
SK9937	rneA1018::bla thyA715 rph-1 recA56 srl-300::TnlO Tc^r/ pM0K13 (rne-1 Cm^1)	Ow et al. (2000)
SK9950	rneA1018::bla thyA715 rph-1 recA56 srl-300::TnlO Tc^r/pFMK33 (rneA225 Km^1)	Ow et al. (2000)
SK9971	rneA1018::bla thyA715 rph-1 recA56 srl-300::TnlO Tc^r/pM0K16 (rneA374 Km^1)	Ow et al. (2000)
SK9957	rneA1018::bla thyA715 rph-1 recA56 srl-300::TnlO Tc^r/pMOK15 (rneA610 Cm^1)	Ow et al. (2000)