Microbiological Research 207 (2018) 134–139

Contents lists available at ScienceDirect

Microbiological Research

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

The special existences: nanoRNA and nanoRNase

Hebin Liao[a], Mafeng Liu[b,1], Xiaolan Guo[a,c,d,*]

[a] Translational Medicine Research Center, North Sichuan Medical College, Nanchong, Sichuan, 637000, PR China
[b] Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Chengdu, Sichuan, 611130, PR China
[c] Department of Laboratory Medicine, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, 637000, PR China
[d] Department of Laboratory Medicine, North Sichuan Medical College, Nanchong, Sichuan, 637000, PR China

ARTICLE INFO

Keywords:
nanoRNA
Orn
NrnA
NrnB
NrnC

ABSTRACT

To adapt to a wide range of nutritional and environmental changes, cells must adjust their gene expression profiles. This process is completed by the frequent transcription and rapid degradation of mRNA. mRNA decay is initiated by a series of endo- and exoribonucleases. These enzymes leave behind 2- to 5-nt-long oligoribonucleotides termed "nanoRNAs" that are degraded by specific nanoRNases; the degradation of nanoRNA is essential because nanoRNA can mediate the priming of transcription initiation that is harmful for the cell via an unknown mechanism. Identified nanoRNases include Orn in *E. coli*, NrnA and NrnB in *B. subtilis*, and NrnC in *Bartonella*. Even though these nanoRNases can degrade nanoRNA specifically into mononucleotides, the biochemical features, structural features and functional mechanisms of these enzymes are different. Sequence analysis has identified homologs of these nanoRNases in different bacteria, including Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Firmicutes and Cyanobacteria. However, there are several bacteria, such as those belonging to the class Thermolithobacteria, that do not have homologs of these nanoRNases. In this paper, the source of nanoRNA, the features of different kinds of nanoRNases and the distribution of these enzymes in prokaryotes are described in detail.

1. The main sources of nanoRNA: mRNA decay

mRNA is one of the most structurally and functionally complex classes of macromolecules in living organisms. mRNA metabolism includes all the processes required for RNA synthesis, maturation, and degradation in living cells (Condon, 2007). Inside cells, mRNAs are frequently transcribed and rapidly degraded to adapt to changing environments (Arraiano et al., 2010). For example, disruptions in mRNA turnover have been linked to pathological processes such as inflammation, cancer and Alzheimer's disease (Nelersa, 2009).

In prokaryotes, mRNA degradation is initiated by endoribonucleases that cleave the mRNA into two smaller fragments. Then, the fragments serve as substrates for further digestion by exoribonucleases (Condon, 2007) (Fig. 1). In the model organism *E. coli*, the essential endoribonuclease RNase E and other minor endoribonucleases, including RNase G, RNase III, RNase P and RNase Z, are responsible for the initial degradation of mRNA (Condon, 2007; Arraiano et al., 2010). RNase E binds to substrates with 5′-monophosphates and endonucleolytically cleaves the RNA downstream of the 5′-terminal AU-rich regions. The new RNA fragments do not have protected 3′-termini; hence, these fragments can be easily cleaved by exoribonucleases, primarily RNase II, PNPase and RNase R (Vincent and Deutscher, 2006). These enzymes can degrade RNA in the 3′-terminal to the 5′-terminal direction into extremely small, 2- to 5-nt fragments known as nanoRNA. Finally, Orn is required to thoroughly degrade nanoRNA into mononucleotides (Niyogi and Datta, 1975a,b) and complete the process of mRNA decay.

On the other hand, in the gram-positive bacteria *B. subtilis*, there are two mRNA decay pathways (Condon, 2003; Lehnik-Habrink et al., 2012; Rische-Grahl et al., 2014). The major pathway is one where mRNAs are initially degraded by RNase Y via endonucleolytic cleavage and further degradation is dependent on RNase J1 (in the 5′-terminal to 3′-terminal direction) and PNPase (in the 3′-terminal to 5′-terminal direction). The other pathway is RNase J1 dependent. RNase J1 can degrade mRNA in the 5′-terminal to 3′-terminal direction. In addition, RNaseJ1 initiates mRNA degradation by endonucleolytic cleavage, and then RNase J1 and PNPase further degrade the fragments as mentioned above (Condon, 2003; Oussenko et al., 2005; Lehnik-Habrink et al., 2012). Small, 2- to 5-nt RNA fragments known as nanoRNAs are produced, and finally, the nanoRNAs are degraded into mononucleotides by NrnA, NrnB or an unidentified enzyme (Mechold et al., 2007). The

* Corresponding author at: Translational Medicine Research Center, North Sichuan Medical College, Nanchong, Sichuan, 637000, PR China.
E-mail address: alan5200@hotmail.com (X. Guo).
[1] Co-first author.

https://doi.org/10.1016/j.micres.2017.11.014
Received 12 July 2017; Received in revised form 23 November 2017; Accepted 25 November 2017
Available online 29 November 2017
0944-5013/

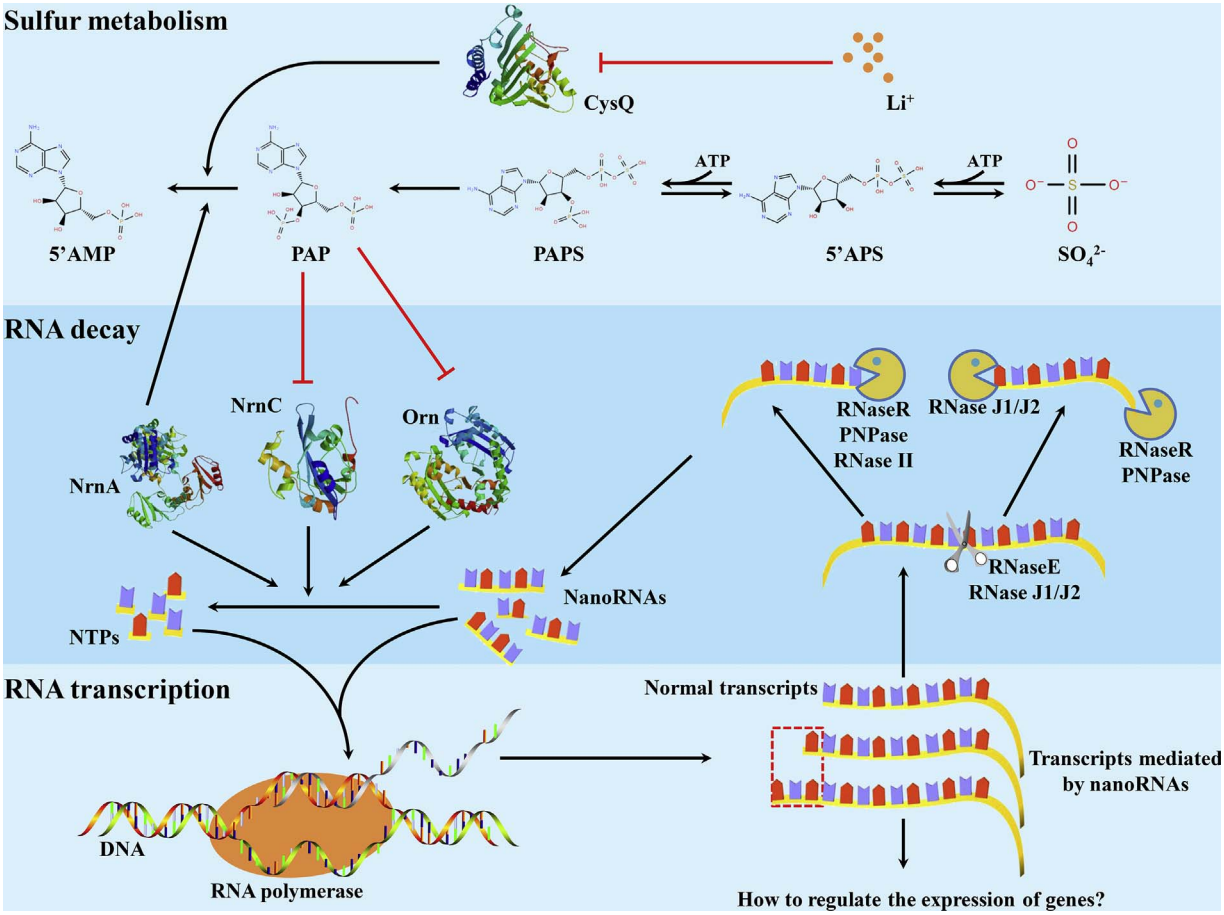

Fig. 1. The relationship between sulfur metabolism, RNA decay and RNA transcription. This figure consists of three parts: sulfur metabolism, RNA decay and RNA transcription, which are indicated by different backgrounds; each component is labeled accordingly. Black arrows indicate synthesis, degradation or activation. Red arrows indicate inhibition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

main RNase enzymes involved in mRNA degradation in bacteria are shown in Table 1.

In addition to its production from the normal degradation of mRNA, nanoRNA can also be produced by other processes. For example, in the process of transcriptional proofreading, the mistranscribed RNA can be degraded by endonucleases into nanoRNA (Zenkin et al., 2006). Additionally, in the process of transcriptional extension, some unknown mechanisms stop transcription and start a new transcription process so that nanoRNA may be produced (Surratt et al., 1991; Borukhov et al., 1992; Fish and Kane, 2002; Zenkin et al., 2006). Moreover, a 2- to 15-nt failure product is synthesized and released during transcriptional initiation *in vitro*; this kind of fragment could be a putative nanoRNA source *in vivo* (Goldman et al., 2009).

2. The function of nanoRNA: mediating the priming of transcription initiation

NanoRNAs are mainly produced during the normal metabolism of mRNA, and whether nanoRNAs play an important role in cell biology is currently being studied. Previous investigations have demonstrated that the interruption of nanoRNA degradation leads to a cessation of the growth of *E. coli*, *S. coelicolor* and *S. griseus* (Ghosh and Deutscher, 1999; Ohnishi et al., 2000; Sello and Buttner, 2008). This finding indicated that the accumulation of nanoRNA is harmful for the growth of bacteria. Ghosh hypothesized that the accumulation of nanoRNA inhibited RNA metabolism by decreasing the concentration of mononucleotides, by inhibiting the activity of an enzyme, or by interfering with an essential metabolic process (Ghosh and Deutscher, 1999).

Because RNA polymerases of prokaryotes and eukaryotes can use 2- to 8-nt RNAs to prime transcription initiation *in vitro* (Smagowicz and Scheit, 1978; Grachev et al., 1984; Ruetsch and Dennis, 1987), and because transcription is traditionally thought to be initiated by NTPs alone, Goldman et al. proposed that nanoRNAs may be able to serve as primers to initiate transcription *in vivo* by competing with NTPs. To prove this hypothesis, Goldman et al. sequenced the 5′ ends of primary transcripts isolated from cells of *P. aeruginosa* that overproduced nanoRNA, identified bona fide promoters and analyzed the shifts in the transcription start sites (Goldman et al., 2011). The results indicated that nanoRNAs were able to prime transcription initiation *in vivo* and caused widespread shifts in transcription start sites to upstream positions. More specifically, the 5′-terminus of the nanoRNA was complementary to positions −3 and +1, and the 3′-terminus was complementary to positions +1, +2 or +3; therefore, the position of the transcription initiation site was shifted (Fig. 1). To investigate the influence of the shift in the transcription start site due to nanoRNA priming of transcription initiation, microarray analysis was used to compare the global gene expression profile of nanoRNA-overproducing cells to that of normal cells; a global alteration in gene expression was observed. For example, the accumulation of nanoRNA can regulate the expression levels of *tomB* and *bhsA* (Zhang et al., 2007; Garcia-Contreras et al., 2008; Mermod et al., 2012; Vvedenskaya et al., 2012).

The mechanisms by which nanoRNA activity influences gene expression are not fully understood now. Here, some assumptions are addressed. One hypothesis is that nanoRNA-mediated transcription could alter the stabilities of transcripts by altering the phosphorylation status of 5′-terminal of primary transcript (Mackie, 1998; Deana et al., 2008; Goldman et al., 2011). Another is that promoters may be significantly affected by altering the sequence of 5′-terminal (Goldman