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Ribonucleases, antisense RNAs and the control of bacterial plasmids

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ABSTRACT

In the last decade regulatory RNAs have emerged as powerful tools to regulate the expression of genes both in prokaryotes and in eukaryotes. RNases, by degrading these RNA molecules, control the right amount of the regulatory RNAs, which is fundamental for an accurate regulation of gene expression in the cell. Remarkably the first antisense RNAs identified were plasmid-encoded and their detailed study was crucial for the understanding of prokaryotic antisense RNAs. In this review we highlight the role of RNases in the precise modulation of antisense RNAs that control plasmid replication, maintenance and transfer.

1. Introduction

Bacteria have evolved complex regulatory networks in order to rapidly adjust their physiology in face of changing environmental conditions. A fine-tuned control of RNA levels ensures survival when a rapid adaptation is essential. RNA degradation is thus fundamental in the regulation of the proper expression of the genetic information, and ribonucleases (RNases), the enzymes that degrade RNA, are key factors in the maintenance of the right amount of each transcript in the cell. RNases are divided into two main classes: endoribonucleases, which cleave RNA internally and exoribonucleases that digest RNA from one extremity. The conventional model for RNA degradation in *Escherichia coli* usually begins with an endonucleolytic cleavage at one or more internal sites on the RNA molecule (Carpousis et al., 2009). Two endoribonucleases have been associated with the initial cleavage events: RNase III, which is specific for double-stranded RNA, and RNase E that cleaves single-stranded molecules (reviewed by Arraiano et al., 2010; Silva et al., 2011). This ribonuclease displays higher activity over substrates bearing a monophosphorylated 5′-end than over substrates with a triphosphorylated 5′-end (Mackie, 1998). After the initial cleavage step the fragments generated are often more sensitive to the action of ribonucleases, and are commonly further degraded by exoribonucleases. In *E. coli* three exoribonucleases are mainly involved in RNA decay: PNPase, RNase R and RNase II. All these enzymes degrade RNA processively and nonspecifically from the 3′ end (Andrade et al., 2009). The action of these enzymes is often stimulated by the addition of poly(A) tails to the 3′ end of the RNA molecules (Regnier and Hajnsdorf, 2009).

Bacillus subtilis does not possess RNase E and instead a functional equivalent endoribonuclease has been identified, RNase Y (Shahbabian et al., 2009). This is also a common characteristic of the low G/C content Gram-positive bacteria. These bacteria, besides having 3′ specific exoribonucleases, also possess RNases J1 and J2, which degrade RNA from the 5′ end (Mathy et al., 2007). RNases J1 and J2 have also the ability to cleave single-stranded RNA internally and are thus classified as endoribonucleases as well (Even et al., 2005). Different RNases may cooperate in the degradation of RNAs, and some of them are found together in a degradative complex called degradosome (Carpousis et al., 1994). This complex brings together all the

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degrading activities necessary to achieve full degradation of an RNA molecule.

It has become clear that antisense RNA molecules can act as functional regulators, and sense/antisense RNA pairing has emerged as a global regulatory mechanism to control RNA levels (Saramago et al., 2014). However, the first regulatory RNAs to be discovered were plasmid encoded. In 1981, Tomizawa and colleagues showed that the antisense RNA RNAI controls the copy number of *E. coli* plasmid ColE1 (Tomizawa et al., 1981). That same year the CopA RNA, involved in the copy number control of *E. coli* plasmid R1, was identified (Stougaard et al., 1981). The antisense Sok RNA of plasmid R1 was then shown to be involved in plasmid stability (Gerdes et al., 1986). Antisense RNAs are now known to be principal regulators of replication, stability and conjugation in plasmids. Their role in modulating lysis/lysogeny in phages and in the control of transposition is also well established. Much was learned about antisense RNA regulation from the detailed studies of these systems. RNases, by being crucial elements in the modulation of the antisense RNA levels, were shown to be key players in the regulation of these processes.

In this minireview, we emphasize the role of cellular RNases and describe how the timed and controlled activity of these enzymes is critical for the antisense RNA regulation of replication, segregational stability and conjugation of plasmids.

2. Role of RNases in the regulation of plasmid replication control

The presence of plasmids not only can bring advantages to the bacterial host cell (like drug resistance genes), but they also constitute a burden for the host. Therefore they have systems to control their copy number in order to ensure that a constant level is maintained in the cell. There are two types of plasmid copy number control systems: the iteron-mediated control, in which the replication protein (Rep) binds to iterons (directly repeated sequences), where it can act as an initiator of plasmid replication or as an inhibitor of overreplication; and antisense RNA-mediated control, in which these RNA elements hybridize to a complementary region of an essential RNA for plasmid replication (Brantl, 2002; Chattoraj, 2000). Antisense RNAs have been considered the main regulators of plasmid copy number, and will be the focus of this section together with other important players, ribonucleases (Table 1). These enzymes have the capability to regulate replication frequency of plasmids by controlling the levels of antisense RNAs and other important RNA transcripts (Wagner and Simons, 1994). Therefore, RNA turnover plays a pivotal role in the control of replication and plasmid copy number. The role of RNases is reinforced by the fact that these antisense RNA elements are usually unstable and their levels accurately reflect the plasmid copy number. The exception is the long-lived antisense RNAIII of the pIP501 plasmid (Brantl and Wagner, 1996), for which a second control element (repressor CopR) is needed for proper regulation (detailed in this special issue by S. Brantl).

2.1. Regulation of ColE1 copy number

ColE1 plasmids were the first found to replicate under the control of an antisense RNA (Tomizawa et al., 1981). It was later found that RNases have a crucial role for the regulation of ColE1 copy number. For this reason ColE1 plasmid is still considered a model for the study of RNA degradation.

In contrast to other plasmids, ColE1 replication does not require a plasmid-encoded Rep protein, but requires the RNA preprimer RNAII synthesized by host RNA polymerase (Fig. 1A). RNAII forms a hybrid with DNA template in the

Table 1
Ribonucleases involved in the control of bacterial plasmids (see text for details).

	Ribonuclease	Mechanism of action
Plasmid copy number		
ColE1 plasmid	RNase E	Initial cleavage of RNAI
	PNPase	3′–5′ degradation of RNAI intermediate products (previously polyadenylated)
	RNase III	Role on RNAI decay (independently of RNAII hybridization)
Plasmid R1	RNase E	Internal cleavage of CopA
	PNPase	3′–5′ degradation of products generated by RNase E cleavage (previously polyadenylated)
	RNase II	
	RNase III	Degradation of CopA-*repA* mRNA duplex
ColE2 plasmid	RNase E	Initial cleavage of RNAI
	PNPase	Subsequent degradation of RNAI intermediate products (previously polyadenylated)
	RNase II	
Plasmid maintenance		
Hok/Sok system of R1	RNase E	Initial cleavage of Sok RNA
	PNPase	3′–5′ degradation of products generated by RNase E cleavage (previously polyadenylated)
	PNPase	Maturation of *hok* mRNA to a translatable conformation able to bind Sok
	RNase II	
	RNase III	Degradation of Sok-*hok* duplex
par System of pAD	RNase J1/J2	Putative role in RNAI cleavage
Plasmid conjugation		
F plasmid	RNase E	FinP cleavage (in the absence of FinO protein) Enhancement of conjugation
	RNase III	Degradation of *traJ*-FinP duplex (in the presence of FinO protein) Repression of conjugation
pCF10 plasmid	RNase III	Processing of *prgX* mRNA-Qs duplex

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