Nucleotide methylations in rRNA that confer resistance to ribosome-targeting antibiotics

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Abstract

Methylation of rRNA nucleotides is an effective means of conferring resistance to antibiotics that target the bacterial ribosome. This type of resistance seems to have evolved as self-defence mechanisms in bacteria such as *Streptomyces* species that synthesize ribosome-targeting drugs. The self-defence mechanisms were subsequently recruited by pathogenic bacteria including streptococcal and staphylococcal species, where resistance to macrolides and related drugs is now a prevalent clinical problem. In this article, we review the methylation events in bacterial rRNA that confer resistance, and discuss how the molecular mechanisms of resistance can be explained from the recent crystal structures of antibiotics bound to the ribosome.

1 Introduction

Many clinically important antibiotics inhibit the growth of bacteria by blocking protein synthesis on the ribosomes (Vázquez 1979; Gale et al. 1981; Spahn and Prescott 1996). These antibiotics bind to regions of the ribosome that are concerned with essential steps in protein synthesis such as peptide bond formation, GTP hydrolysis and mRNA decoding. The main contact sites for the antibiotics are on the rRNA, rather than on the ribosomal protein components (Cundliffe 1990), which is consistent with the view that the rRNA carries out the primary functions of the ribosome, including the formation of the peptide bond (Green and Noller 1997; Nissen et al. 2000). Not surprisingly therefore, changes in the ribosome structure that confer antibiotic resistance are mainly to be found in the rRNA, and consist of nucleotide methylations or base substitutions (Cundliffe 1990). There are indeed cases of ribosomal protein (r-protein) mutations which confer resistance to ribosome-targeting antibiotics in laboratory (Cundliffe 1990; Chittum and Champney 1994; Belova et al. 2001; Kofoed and Vester 2002; Bosling et al. 2003) plus veterinary and clinical strains (Aarestrup and Jensen 2000; Adrian et al. 2000b; McNicholas et al. 2000; Tait-Kamradt et al. 2000; Farrell et al. 2003). However, these r-protein mutations tend to confer resistance in an indirect manner by influencing the conformation of adjacent rRNA structures that

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make contact with the antibiotic (Gregory and Dahlberg 1999; Gabashvili et al. 2001).

In this review, we concentrate on rRNA methylations (Fig. 1) that are directed by specific methyltransferase enzymes and confer resistance to ribosome-targeting drugs (Table 1). This form of resistance is found in many pathogenic and drug-producing bacteria and tends to be absent in other bacterial species. The resistance methyltransferases seem to have no function in the absence of the antibiotic against which they offer protection; indeed, in many cases these are expressed only when the antibiotic is present. This contrasts with the many other modifications in rRNAs that are present under most if not all growth conditions and carry out 'house-keeping' roles important for the general functioning of rRNA during protein synthesis.

2 Ribosomal RNA modifications

Bacterial rRNAs can contain over thirty house-keeping modifications all of which are added post-transcriptionally. The sites and types of modification have been most accurately mapped in *Esherichia coli,* where 16S and 23S rRNAs contain eleven and twenty-four modifications, respectively, that consist of pseudouridinylations, base methylations, and the 2′-O-methylation of riboses (Rozenski et al. 1999; Andersen et al. 2004). These rRNA modifications are present under most growth conditions, and modification is generally the product of a specific enzyme encoded by a gene inherent in the bacterial chromosome. Each resistance methyltransferase is also encoded by a specific gene. In actinomycetes, resistance genes are generally an integral part of a chromosome region concerned with antibiotic production, whereas in pathogenic bacteria resistance genes are often acquired on plasmids or transposons.

The requirement in bacteria for a specific enzyme for each modification contrasts with the mechanisms in eukaryotic cells. Pseudouridinylation reactions and 2′-O-methylations, which make up the bulk of eukaryotic rRNA modifications, are guided by a variety of snoRNAs that function together with a limited set of enzymes (Kiss 2002; Ofengand 2002; Decatur and Fournier 2003). An overview of eukaryotic rRNA modification is provided by Yu et al. in this volume.

The locations of the rRNA modifications can be charted on the crystallographic structures of the ribosome (Ban et al. 2000; Schlünzen et al. 2000; Wimberly et al. 2000; Harms et al. 2001; Yusupov et al. 2001), and cluster in regions concerned with the essential ribosome functions (Brimacombe et al. 1993; Ofengand and Rudd 2000; Decatur and Fournier 2002). For the most part, the post-transcriptional modifications fine-tune the function of rRNA in protein synthesis, and this has been demonstrated by the superior performance of authentic rRNAs compared to their unmodified 16S (Krzyzosiak et al. 1987) and 23S counterparts (Green and Noller 1999; Khaitovich et al. 1999). Although the resistance methyltransferases only benefit rRNA function when the bacterium is challenged by an antibiotic, the advantage afforded by a resistance methylation under these condi-