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An aminoglycoside sensing riboswitch controls the expression of aminoglycoside resistance acetyltransferase and adenyltransferases ☆

Dongrong Chen [a,b,*], Alastair I.H. Murchie [a,b,c,*]

[a] Key Laboratory of Molecular Medicine, Ministry of Education, Department of Biochemistry and Molecular Biology, Fudan University Shanghai Medical College, Shanghai 200032, PR China
[b] Institutes of Biomedical Sciences, Fudan University Shanghai Medical College, Shanghai 200032, PR China
[c] School of Pharmacy, Fudan University, Zhang Heng Road 826, Pudong 201203, Shanghai, PR China

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ABSTRACT

The emergence of antibiotic resistance in human pathogens is an increasing threat to public health. The fundamental mechanisms that control the high levels of expression of antibiotic resistance genes are not yet completely understood. The aminoglycosides are one of the earliest classes of antibiotics that were introduced in the 1940s. In the clinic aminoglycoside resistance is conferred most commonly through enzymatic modification of the drug although resistance through enzymatic modification of the target rRNA through methylation or the overexpression of efflux pumps is also appearing. An aminoglycoside sensing riboswitch has been identified that controls expression of the aminoglycoside resistance genes that encode the aminoglycoside acetyltransferase (AAC) and aminoglycoside nucleotidyltransferase (ANT) (adenyltransferase (AAD)) enzymes. AAC and ANT cause resistance to aminoglycoside antibiotics through modification of the drugs. Expression of the AAC and ANT resistance genes is regulated by aminoglycoside binding to the 5′ leader RNA of the *aac/aad* genes. The aminoglycoside sensing RNA is also associated with the integron cassette system that captures antibiotic resistance genes. Specific aminoglycoside binding to the leader RNA induces a structural transition in the leader RNA, and consequently induction of resistance protein expression. Reporter gene expression, direct measurements of drug RNA binding, chemical probing and UV cross-linking combined with mutational analysis demonstrated that the leader RNA functioned as an aminoglycoside sensing riboswitch in which drug binding to the leader RNA leads to the induction of aminoglycoside antibiotic resistance. This article is part of a Special Issue entitled: Riboswitches.

1. Introduction

The aminoglycoside antibiotics were among the earliest of the antibiotic classes to be used to combat serious bacterial infections (reviewed in [1,2]). They cause mistranslation of mRNA and inhibit ribosomal translocation by interacting with rRNA in the decoding region of the 30S ribosomal subunit at the A site [3–5]. They are natural products, and new aminoglycosides have been steadily developed to target the proliferation of antibiotic resistant infections [1]. Like other natural product antibiotics, the aminoglycosides are secondary metabolites. Their biosynthesis involves multiple gene products and complex biosynthetic pathways [6]. They rarely achieve the dosing levels necessary for antibiotic activity within their natural producer organism. For this reason it is probably naive to regard them as mere inhibitors of bacterial growth; they have been predicted to act as regulatory effector molecules for a range of biological activities [7]. Novel functionalities for antibiotics continue to emerge such that they can be considered to be modulators as well as inhibitors [2,6,8].

The cationic amine groups of the aminoglycosides bind to negatively charged pockets in structured RNA [9] and additional binding sites have been found in rRNA [5,10]. Aminoglycoside binding sites have been identified on a number of RNAs; and include the HIV trans-activating-region [11] and Rev responsive element [12] and autocatalytic ribozymes [13]. Aminoglycoside binding is not simply linked to general inhibitory activities [8]; they promote self-cleavage by the hairpin ribozyme in the absence of metal ions [14] whereas they inhibit hammerhead ribozyme activity [15]. Sub-inhibitory doses of tobramycin induce biofilm formation in *Pseudomonas aeruginosa* and in *Escherichia coli* [16]. Here, rather than targeting the bacterial ribosome, the antibiotic targets a putative cyclic diguanosine monophosphate (c-di-GMP) phosphodiesterase (the aminoglycoside response regulator gene (*arr*)) leading to the induction of biofilm-specific aminoglycoside resistance. Cyclic diguanosine monophosphate is a second messenger molecule that controls cell surface adhesion [16]. The bacterial SOS response is also induced by sub-maximal doses of antibiotics (including aminoglycosides) through interference with the SOS transcriptional repressor LexA [17,18].

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* Corresponding authors at: Institutes of Biomedical Sciences, Fudan University Shanghai Medical College, Shanghai 200032, PR China. Tel.: +86 21 54237517; fax: +86 21 54237577.
E-mail addresses: drchen@fudan.edu.cn (D. Chen), aihm@fudan.edu.cn (A.I.H. Murchie).

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The capability of the aminoglycosides to shape RNA structures has been used to engineer aminoglycoside binding RNA aptamers [19,20]. A novel binding mode associated with an *in vitro* selected streptomycin binding RNA aptamer [20] led to the notion that natural aminoglycoside binding aptamers may have regulatory roles [21,22]. Through a combination of *in vitro* selection and genetic screening a neomycin responsive synthetic riboswitch was identified that could regulate translational initiation in *Saccharomyces cerevisiae* [23,24].

Resistance to aminoglycosides emerged soon after they were introduced and is associated with the mobile elements on plasmids or integrons responsible for transmissible multi-drug resistance [25]. Integrons were originally discovered through the proliferation of antibiotic resistance (reviewed in [26]). Integrons accumulate antibiotic resistance genes through site-specific recombination at a conserved DNA sequence (*attI1*) that is the target for the integron encoded integrase (int) [27–29]. Aminoglycoside resistance occurs through the overexpression of efflux pumps or by enzymatic methylation of the target 16S rRNA or modification of the drug [30]. The drugs are inactivated by the activity of resistance enzymes at specific positions on the molecule; by acetyltransferases (N-acetylation (classified as AAC)), nucleotidyltransferases or adenyltransferases (O-adenylylation (ANT, also classified as AAD by Jia and colleagues [31])), or phosphotransferases (O-phosphorylation (APH)) (Fig. 1A) [32]. Resistance to the aminoglycosides can be induced by the presence of the drugs [32–34]. The mechanisms of induction of resistance genes by translational inhibitors have been studied for several decades [35–38]. The best understood system of induction of resistance to an antibiotic that acts on the ribosome is that of induction of the erythromycin resistance methyltransferase *ermC*. The *ermC* system exploits

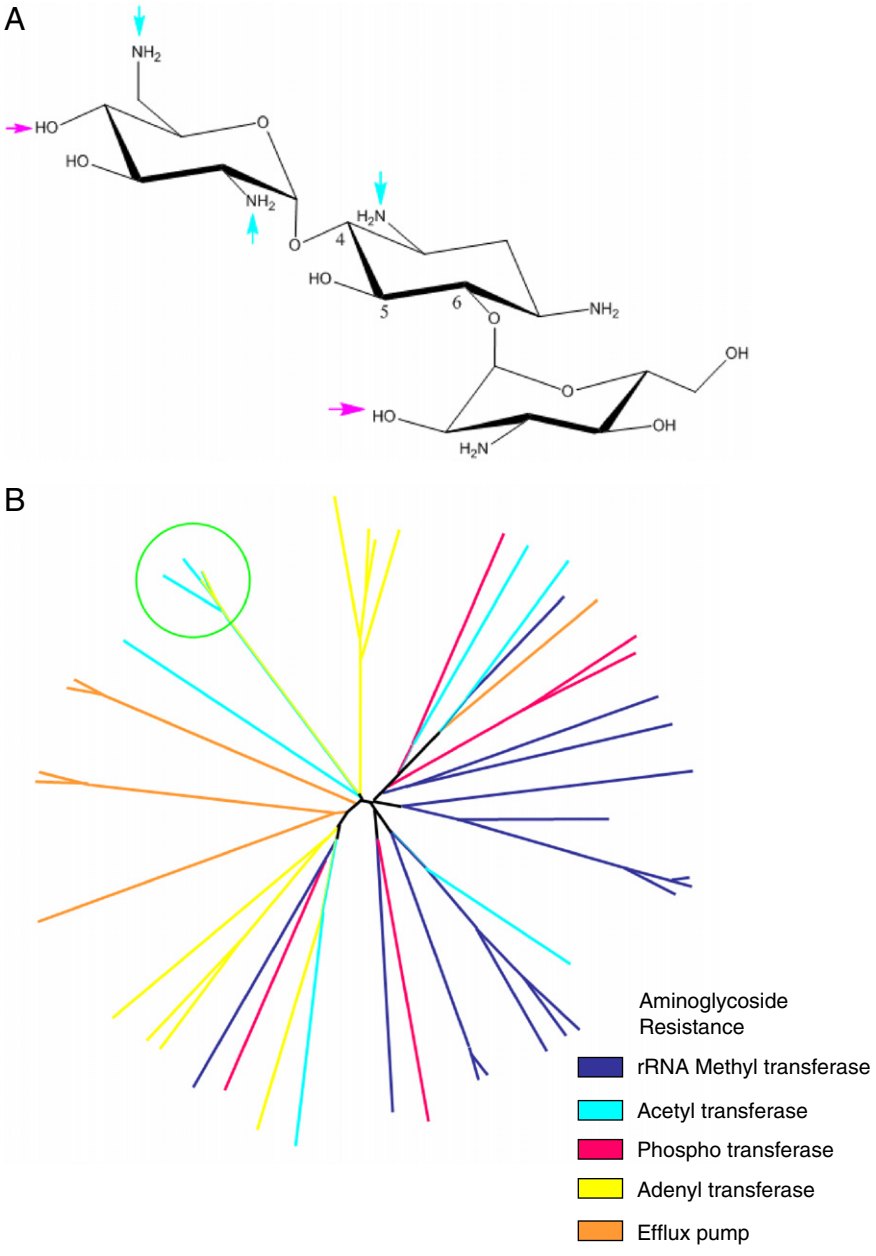

Fig. 1. (A) Kanamycin B, the 4,6 deoxystreptamine aminoglycoside antibiotic that is most susceptible to inactivation by enzymatic modification. The 4, 5 and 6 positions of deoxstreptamine are indicated. Arrows indicate the major sites of inactivation by N-acetylation (cyan) and O-adenylation (magenta). (B) Multiple sequence alignment of the leader RNA sequence; unrooted dendrogram of 50 aminoglycoside resistance genes including genes encoding AAC (cyan), APH (red), ANT (yellow), rRNA methyltransferases (blue) or efflux pumps (orange); for key, see Table S1. Two highly conserved AAC and three AAD genes are circled.

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