Research in Microbiology 169 (2018) 61–66

Contents lists available at ScienceDirect

Research in Microbiology

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

Review

The *cfr* and cfr-like multiple resistance genes

Birte Vester

Dept. of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark

ARTICLE INFO

Article history:
Received 5 October 2017
Accepted 11 December 2017
Available online 2 March 2018

Keywords:
Cfr
Cfr-like enzymes
Antibiotic resistance
RNA methylation

ABSTRACT

The Cfr methyl transferase causes an RNA methylation of the bacterial ribosomes impeding reduced or abolished binding of many antibiotics acting at the peptidyl transferase center. It provides multi-resistance to eight classes of antibiotics, most of which are in clinical and veterinary use. The *cfr* gene is found in various bacteria in many geographical locations and placed on plasmids or associated with transposons. Cfr-related genes providing similar resistance have been identified in Bacillales, and now also in the pathogens *Clostridium difficile* and *Enterococcus faecium*. In addition, the presence of the *cfr* gene has been detected in harbours and food markets.

1. Introduction

There is an increasing concern about pathogenic bacteria obtaining resistance to the antibiotics used for treatment of humans and animals. To address the problems with antibiotic resistance we need knowledge about the various resistance mechanisms, how they can be disseminated, which bacteria contain them and where they come from. Antibiotic resistance is not a new phenomenon, see e.g. review by [1]. There are many ways bacteria can obtain and exert antibiotic resistance and new resistance determinants are continuously discovered. The strategies bacteria use to evade the effects of antibiotics, can be grouped into three general mechanisms: increased antibiotic efflux out of the cell or reduced antibiotic influx into the cell, enzymatic inactivation of antibiotics through drug modification or cleavage, and the alteration of the antibiotic binding site. A bacterial cell contains many different target sites for antibiotics, but in general, an antibiotic interfere with or inhibits an essential cellular pathway or process. The most common antibiotic targets include bacterial cell wall synthesis; the bacterial membrane; the DNA replication machinery; RNA polymerase; the folate biosynthesis pathway; and the protein synthesis machinery.

The ribosome is a major site of antibiotic action in the bacterial cell and is targeted by a large and chemically diverse group of antibiotics. A number of these antibiotics have important applications in human and veterinary medicine in the treatment of bacterial infections. The antibiotic binding sites are clustered at functional centers of the ribosome, such as the decoding center on the 30S subunit, the peptidyl transferase center (PTC), the GTPase center, and the peptide exit tunnel on the 50S subunit and the subunit interface in the 70S ribosome. The resistance mechanisms to antibiotics targeting the ribosomes include all three mechanism mentioned above. The alterations of the ribosomal antibiotic binding sites providing antibiotic resistance can be either mutation or methylation. While mutations appear spontaneously the gaining of methylations normally need a gene transfer of a methyl transferase gene. Since the 1950s, about 17 methyltransferases providing antibiotic resistance has been discovered, reviewed by [2,3]. One of the most recently discovered and most exceptional regarding nature of modification and mechanism of methylation is the Cfr methyltransferase [4–7] and Cfr and Cfr-like proteins have a potential to become a serious threat for antibiotic treatment. The sections below will present an overview of our current knowledge about Cfr and Cfr-like methyltransferases and especially cover aspects that have not previously been reviewed.

2. The Cfr methyltransferase and its target

The *cfr* gene codes for Cfr, a 349 amino acid long RNA methyltransferase, characterized as a radical SAM methyltransferase [8]. Cfr makes one methylation at the bacterial ribosome, causing reduced or abolished binding of many antibiotics that bind to the peptidyl transferase center (PTC) of the bacterial ribosomes [5] (Fig. 1A). Many different antibiotics act by binding to the ribosomes where their binding inhibits peptide synthesis and thereby bacterial growth [9]. Chemically different antibiotics can bind at the same specific sites in the ribosomes, although not totally

E-mail address: b.vester@bmb.sdu.dk.

https://doi.org/10.1016/j.resmic.2017.12.003
0923-2508/

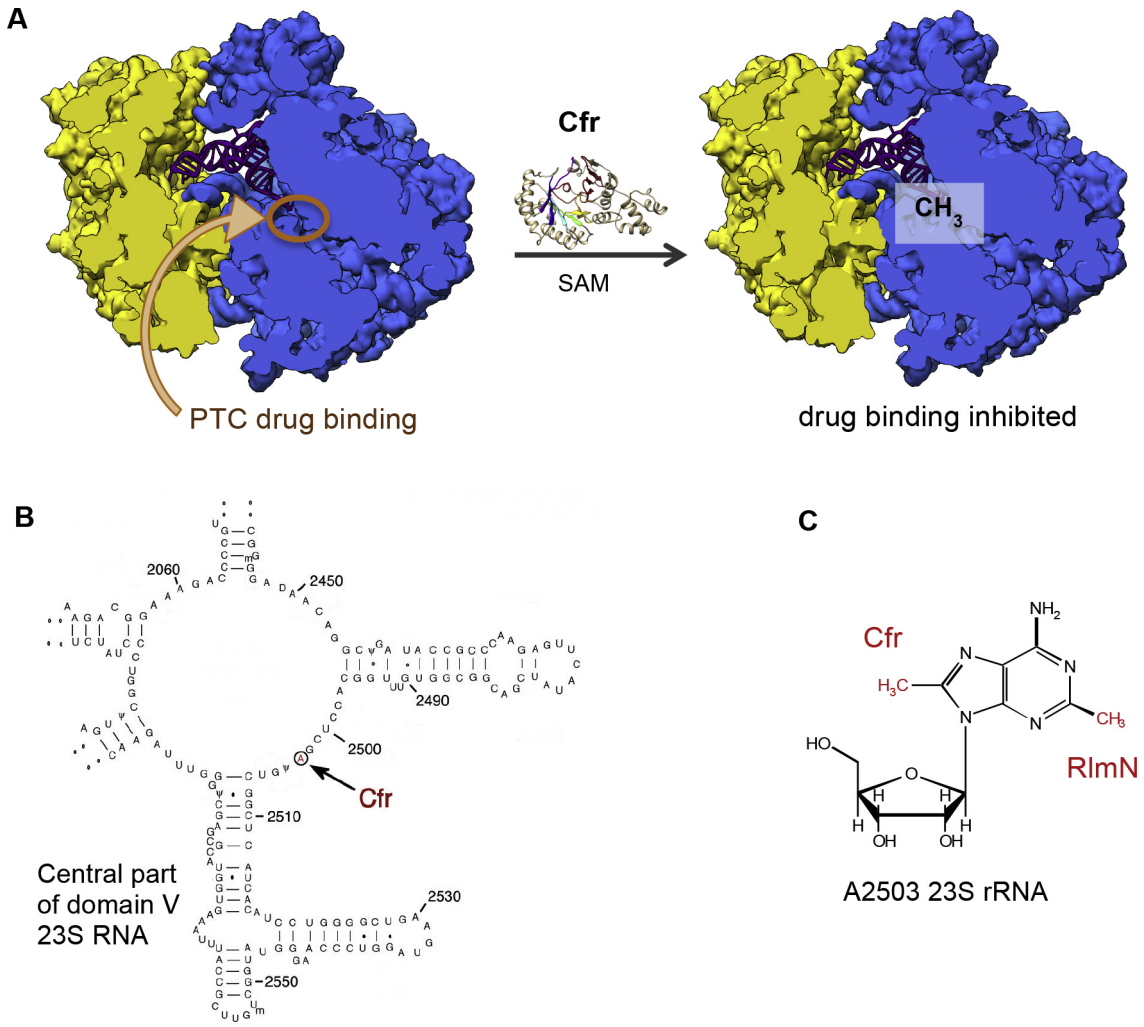

Fig. 1. The Cfr methylation site in the ribosome. A: A cut view of the *E. coli* ribosome with tRNA in P-site (purple) (PDB 4V9D) with a circle indicating the PTC where Cfr methylates A2503 23S RNA and thereby inhibits binding of the antibiotics at this region. B: central part of domain V 23S RNA constituting part of the PTC. The arrow points at position A2503 where Cfr adds a methyl group. C: the chemical structure of the double methylated A2503 with the enzymes shown in red. Cfr acts at the C-8 position and RlmN at the C-2 position. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

overlapping. The PTC is such a site and the Cfr enzyme methylates A2503 in 23S ribosomal RNA in the PTC (Fig. 1B and C). This is so far the only adenine nucleotide found to be methylated at the C-8 position [6] and this provides multi-resistance to eight different classes of antibiotics, most of which are in clinical and veterinary use. At its discovery on a plasmid in *Staphylococcus sciuri* [10] the gene was reported to provide resistance to chloramphenicol and florfenicol. A later study [11] showed that Cfr provided resistance to: Phenicols (including Chloramphenicol and Florfenicol), Lincosamides (including Clindamycin), Oxazolidinones (including Linezolid), Pleuromutilins (including Tiamulin and Valnemulin), and Streptogramin A's (including Pristinamycin II, Viginiamycin M, and Dalfopristin), all relevant for use in animals or humans. Since then, Cfr has also been shown to provide resistance to large macrolides [12], Hygromycin A and A201A [13].

3. Which bacteria contain the *cfr* resistance gene and where are they found

Until now, the cfr gene has mainly been identified in strains belonging to *Staphylococcus* but it has also been found in *Enterococcus, Bacillus, Proteus vulgaris, Escherichia coli, Macrococcus caseolyticus, Jeotgalicoccus pinnipedialis,* and *Streptococcus suis* (reviewed in [14–17]). The *cfr* genes only contain small variations of a few amino acids as clearly seen by a BLAST search that shows 99% identity between Cfr's until the identity jumps to 77% and the following sequences must be characterised as Cfr-like proteins (see below). The *cfr* gene is often found on plasmids and if chromosomal it seems always associated with insertion elements. A detailed investigation detected a novel variant of the phenicol resistance transposon Tn558 in *Staphylococcus* isolates that harboured an additional resistance gene region, including the *cfr* gene, integrated into the tnpC reading frame [18]. They detected transpositionally active forms of the IS21-558 element, known as minicircles, and suggest a pathway for mobility of the *cfr* gene. A more recent study analysed the genetic environment of the *cfr* gene in *Staphylococcus* isolates by sequencing of the up- and downstream regions on various plasmid types [19] and found insertion sequences (IS21-558, IS256, IS257, or IS1216E) as well as other resistance genes. In chromosomes they found the *cfr* gene to be bracketed by insertion sequences, such as IS256 or ISEnfa5 and stability tests confirmed that these cfr-containing regions could be looped out via IS-mediated recombination. Other examples can be found in [17,20–22] and references therein. A recent summary of the