Biochemical and Biophysical Research Communications 453 (2014) 254–267

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Review

Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations

Jingjing Sun [1], Ziqing Deng [1], Aixin Yan *

School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong Special Administrative Region

ARTICLE INFO

Article history:
Received 2 May 2014
Available online 27 May 2014

Keywords:
Antibiotics
Multidrug resistance
Multidrug efflux pumps
Regulation
Physiology
Efflux pump inhibitors

ABSTRACT

Multidrug resistance (MDR) refers to the capability of bacterial pathogens to withstand lethal doses of structurally diverse drugs which are capable of eradicating non-resistant strains. MDR has been identified as a major threat to the public health of human being by the World Health Organization (WHO). Among the four general mechanisms that cause antibiotic resistance including target alteration, drug inactivation, decreased permeability and increased efflux, drug extrusion by the multidrug efflux pumps serves as an important mechanism of MDR. Efflux pumps not only can expel a broad range of antibiotics owing to their poly-substrate specificity, but also drive the acquisition of additional resistance mechanisms by lowering intracellular antibiotic concentration and promoting mutation accumulation. Over-expression of multidrug efflux pumps have been increasingly found to be associated with clinically relevant drug resistance. On the other hand, accumulating evidence has suggested that efflux pumps also have physiological functions in bacteria and their expression is subject tight regulation in response to various of environmental and physiological signals. A comprehensive understanding of the mechanisms of drug extrusion, and regulation and physiological functions of efflux pumps is essential for the development of anti-resistance interventions. In this review, we summarize the development of these research areas in the recent decades and present the pharmacological exploitation of efflux pump inhibitors as a promising anti-drug resistance intervention.

Contents

Abbreviations: $3OC_6$-HSL, 3-oxohexanoyl homoserine lactone; $3OC_{12}$-HSL, N-(3-oxododecanoyl)-L-homoserine lactone; ABC, the ATP (adenosine triphosphate)-binding cassette superfamily; ABI-PP, AcrAB/MexAB-specific inhibitor of pyridopyrimidine derivative; AHL, *N*-acylhomoserine lactones; BRC, BmrR C terminus; C_4-HSL, N-butyryl homoserine lactone; CCCP, carbonylcyanide-3-chlorophenylhydrazone; CTD, C-terminal domain; DARPin, designed ankyrin repeat protein; DBD, DNA binding domain; DDM, n-dodecyl-D-maltoside; DMT, drug/metabolite transporter superfamily; DNP, 2,4-dinitrophenol; EPI, efflux pump inhibitor; Eb, ethidium bromide; EMSA, electrophoretic mobility shift assay; Et, ethidium; MATE, the multidrug and toxic compound extrusion family; MDCK, Madin–Darby canine kidney; MDR, multidrug resistance; MFS, the major facilitator superfamily; MIC, minimum inhibitory concentration; NMP, naphthylpiperazines; NP, nature product; Pf, proflavin; PAβN, phenyl-arginine beta-naphthylamide; PQS, 2-heptyl-3-hydroxy-4-quinolone; QS, quorum sensing; RND, the resistance-nodulation-division family; SMR, the small multidrug resistance family; TCS, two component system; TPP, tetraphenylphosphonium.

* Corresponding author. Fax: +852 25599114.
E-mail address: ayan8@hku.hk (A. Yan).
[1] These authors contribute equally.

http://dx.doi.org/10.1016/j.bbrc.2014.05.090
0006-291X/

1. Introduction: drug efflux transporters and their clinical relevance

Efflux pumps are found in almost all bacterial species and genes encoding this class of proteins can be located on chromosomes or plasmids [1,2]. According to their composition, number of transmembrane spanning regions, energy sources and substrates, bacterial efflux pumps are classified into five families: the resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the ATP (adenosine triphosphate)-binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family [a member of the much larger drug/metabolite transporter (DMT) superfamily], and the multidrug and toxic compound extrusion (MATE) family [1–3]. Except for the RND superfamily which is only found in Gram-negative bacteria, efflux systems of the other four families: MFS, ABC, SMR and MATE are widely distributed in both Gram-positive and negative bacteria [4]. Depending on the specific classes they belong to, efflux pumps are either single-component transporters or multiple-component systems containing not only an inner membrane transporter, but also an outer membrane channel and a periplasmic adaptor protein, such as the RND type efflux pumps [5]. Owing to their tripartite composition which allows direct extrusion of various drugs from cytosol or periplasmic space to the outside of bacterial cells, RND family pumps have been found to be associated extensively with clinically significant antibiotic resistance, such as AcrB in *Escherichia coli* and *Salmonella typhimurium* and MexB in *Pseudomonas aeruginosa*. In Gram-positive bacteria, the clinically significant efflux pumps are members of the MFS family, for example NorA in *Staphylococcus aureus* and PmrA in *Streptococcus pneumoniae* [6].

In recent decades, with the development of various molecular approaches [5], such as reverse transcription quantitative PCR (RT-qPCR) and immunoblotting, association of efflux pump overexpression with clinically relevant levels of MDR has been increasingly reported [7]. For instance, a recent screening of 50 clinical *E. coli* strains isolated from human clinical samples and dog feces in Sapporo, Japan, revealed a strong correlation of overexpression of the AcrAB efflux pumps with the high-level fluoroquinolone resistance in all 20 multi-resistant strains [8]. In another screening of 52 *Klebsiella pneumoniae* strains isolated from burn patients hospitalized in Shahid Motahari Hospital, Tehran, all 40 isolates which displayed resistance to ciprofloxacin, tetracycline, ceftazidime and gentamicin were found to express high levels of the AcrAB efflux pump particularly in ciprofloxacin resistant strains [9]. In addition, clinical resistance caused by overexpression of more than one efflux pumps was also identified. For instance, simultaneous overexpression of the MexAB–OprM and MexXY efflux systems was demonstrated to account for the multi-resistance phenotype of a collection of 12 *P. aeruginosa* clinical isolates identified in a hospital in France [10]. A clinical isolate of *Stenotrophomonas maltophilia* strain with high minimum inhibitory concentration (MIC) of several antibiotics was found to coordinately hyper-express the RND family efflux pumps SmeZ and SmeJK [11]. In addition to those encoded on the chromosomes of bacteria, plasmid-encoded efflux pumps, such as OqxAB, which confer resistance to olaquindox, was also found to cause drug resistance in *E. coli* clinical isolates [12]. Association of efflux pump overexpression with clinically relevant MDR in Gram-positive bacteria was also reported. Among several hundred clinical isolates of *S. aureus* studied by Christos Kosmidis et al. it was found that strains overexpressing efflux pump genes were common and were widely distributed geographically. These strains were mainly resistant to methicillin and the resistance was clonally related with *norA* and *mepA* overexpression [13].

2. Mechanisms of drug extrusion by the efflux pumps

Efflux pumps are prominent in terms of both their high efficiency of drug extrusion and broad substrate specificities, underlying their roles in multidrug resistance. Substrate profile of the *E. coli* housekeeping efflux system AcrAB-TolC has been studied and it was shown to include chloramphenicol, fluoroquinolone, tetracycline, novobiocin, rifampin, fusidic acid, nalidixic acid and β-lactam antibiotics [2]. Similar to that in *E. coli*, the AcrAB-TolC efflux system in *S. typhimurium* was also found to be able to expel different classes of antimicrobial agents such as quinolones, chloramphenicol, tetracycline and nalidixic acid [1,14]. In *P. aeruginosa*, two RND efflux pumps, MexAB–OprM which is the homolog of the *E. coli* AcrAB-TolC system and MexXY-OprM, are constitutively expressed and both of the systems can actively export fluoroquinolones, tetracycline and chloramphenicol. In addition to these common substrates, MexAB–OprM system can also export novobiocin and β-lactams, such as carbenicilline, and MexXY system can also export aminoglycosides [15]. Substrate profiles of other clinically relevant pathogens are reviewed elsewhere [6,14].

2.1. Structures of RND efflux pumps

Owing to their prominent roles in MDR, various of biophysical and biochemical characterization of bacterial efflux pumps, especially the *E. coli* AcrAB-TolC system, have been conducted [16–21]. In recent decade, elucidation of crystal structures of several drug efflux pumps and those complexed with the substrates or inhibitors has greatly accelerated our understanding of the fundamental mechanism of drug export and the characteristics of their multisubstrate specificities. The first crystal structure of drug efflux pump was that of the *E. coli* AcrB protein which was resolved at 3.5 Å resolution by Murakami et al. [22]. The crystal was grown in the trigonal space group R32, implying a symmetric AcrB trimer. The trimeric complex is comprised by a large portion of the periplasmic headpiece and a transmembrane region. The upper part of the headpiece forms the TolC docking domain and the center of the headpiece comprises the pore domain. Crystal structure of AcrB with its substrate, minocycline or doxorubicin [23] was resolved subsequently by the same research group. Findings from this co-crystal showed that only one of the three protomers bound with the substrate minocycline or doxorubicin (Fig. 1A). This, combined with the asymmetric structure of AcrB revealed by the X-ray crystal structure obtained independently by other two groups [24,25], led to the proposal of the asymmetric configuration of AcrB