

Microbial Primer: Multidrug efflux pumps

Pauline Ann Siasat and Jessica M. A. Blair*

Abstract

Multidrug efflux pumps are molecular machines that sit in the bacterial cell membrane and pump molecules out from either the periplasm or cytoplasm to outside the cell. While involved in a variety of biological roles, they are primarily known for their contribution to antibiotic resistance by limiting the intracellular accumulation of antimicrobial compounds within bacteria. These transporters are often overexpressed in clinical isolates, leading to multidrug-resistant phenotypes. Efflux pumps are classified into several families based on their structure and understanding the characteristics of each family is important for the development of novel therapies to restore antibiotic potency.

INTRODUCTION

Antibiotics are one of the foundations of modern medicine. For most antibiotics to be effective, a sufficient amount of these drugs must enter and accumulate within bacterial cells. To prevent this, bacteria have evolved several resistance mechanisms to combat antibiotic activity, including multidrug efflux pumps.

Multidrug efflux pumps are protein machines that export substrates from inside cells to the external environment. Following their discovery 30 years ago, these transporters have been established as key drivers of antimicrobial resistance (AMR) because they can pump antibiotic molecules out of bacterial cells (Fig. 1). They serve as the first line of defence against antimicrobials, working in conjunction with the cell membrane of Gram-positive, and the cell envelope of Gram-negative, bacteria. By utilizing energy obtained from either ATP or an electrochemical gradient, these proteins export an extensive range of toxic compounds – including antibiotics, dyes, metals and detergents. While many of these substrates are captured from within the cytoplasm and transported across the inner membrane, in Gram-negative bacteria there are also pumps that remove substrates from the periplasm and export them across the outer membrane.

Efflux pumps contribute to antibiotic resistance by effectively preventing the accumulation of antibiotics inside bacterial cells. Resistance in clinical isolates is further driven by pump overexpression – often resulting in multidrug-resistant (MDR) phenotypes. By providing an intrinsic basal level of resistance to many antibiotics, the overexpression of efflux pumps serves as a platform for the development of other resistance mechanisms.

Found in all domains of life, efflux pumps are classified into six families: (i) ATP-binding cassette (ABC); (ii) major facilitator superfamily (MFS); (iii) multidrug and toxin extrusion (MATE); (iv) small multidrug resistance (SMR); (v) proteobacterial antimicrobial compound efflux (PACE); and (vi) resistance-modulation-cell division (RND) (Fig. 2). Although extensively studied for their role in antibiotic resistance, these transporters are also involved in a variety of physiological functions, including virulence, biofilm formation and cellular metabolism. A combination of efflux pumps from each family are expressed in all species of bacteria, working synergistically to promote fitness and pathogenicity.

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Keywords: antibiotic resistance; multidrug efflux pumps; RND.

Abbreviations: ABC, ATP-binding cassette; AHLs, acyl-homoserine lactones; AIs, autoinducers; AMR, antimicrobial resistance; ATP, adenosine triphosphate; CCCP, carbonyl cyanide m-chlorophenylhydrazone; EPIs, efflux pump inhibitors; MATE, multidrug and toxin extrusion; MDR, multidrug resistance; MFS, major facilitator superfamily; MRSA, methicillin-resistant *Staphylococcus aureus*; NBDs, nucleotide-binding domains; OMF, outer membrane factor; PACE, proteobacterial antimicrobial compound efflux; PAP, periplasmic adaptor protein; PAGN, phenylalanine-arginine-β-naphthylamide; PMF, proton motive force; QACs, quaternary ammonium compounds; QS, quorum sensing; RND, resistance-modulation-cell division; SMR, small multidrug resistance; TMDs, transmembrane domains; TPP, tetraphenylphosphonium.

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Microbial Multidrug Efflux

**Chiaraluce Moretti, Roberto
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Microbial Multidrug Efflux:

Microbial Multidrug Efflux Ian T. Paulsen, Kim Lewis, 2002 Resistance of microorganisms to multiple antibiotics and other drugs is a major problem throughout the world Multidrug efflux is a process whereby a single transporter can remove a broad range of substrates from the cell thereby causing resistance to substances such as drugs and antibiotics This book provides the first comprehensive overview of this phenomenon in microorganisms and contains reviews of the current research in this important field With contributions from Internationally renown authors topics covered include genomic analysis of drug efflux pumps the large scale knock out of drug transporters structure function analysis of drug pumps the inhibition of the efflux pump as a clinical approach and much more An essential text for all scientists with an interest in drug and antibiotic resistance in a range of different microorganisms *Efflux-Mediated Antimicrobial Resistance in Bacteria* Xian-Zhi Li, Christopher A. Elkins, Helen I. Zgurskaya, 2016-11-09 This book written by leading international experts provides a comprehensive current examination of transport mediated antimicrobial resistance As a particularly powerful mechanism of multidrug resistance an in depth examination of efflux pumps is conducted with bacteria of major public health concern including Enterobacteriaceae Acinetobacter Neisseria Pseudomonas staphylococci and mycobacteria The content spans structural biochemistry and transport mechanisms of the major transporter families and considers individual drug efflux systems across various Gram positive and Gram negative species Genomic analysis of efflux pump distribution and their contribution to clinically relevant resistance are a major focus of the text Moreover interplay between drug efflux pumps and other key resistance mechanisms such as intrinsic drug impermeability inactivation and target alterations are discussed as well as their molecular expression based regulation and physiological functions beyond resistance involving biofilms stress response and pathogenicity Finally strategies are addressed to target this drug resistance mechanism with novel antimicrobials or drug inhibitor adjuvants Microbial Efflux Pumps Edward W. Yu, Qijing Zhang, Melissa H. Brown, 2013 Infectious diseases caused by bacteria remain a leading cause of death worldwide Many of the antibiotics developed to combat bacterial infections have been rendered almost impotent due to the rapid evolution and spread of antibiotic resistance With contributions from leading researchers in the field this book reviews the most important current research and summarizes the most spectacular discoveries Essential reading for all scientists with an interest in drug and antibiotic resistance in a range of different microorganisms Host-Microbe Interactions, 2016-08-03 Host Microbe Interactions the latest volume in the Progress in Molecular Biology series provides a forum for the discussion of new discoveries approaches and ideas in molecular biology It contains contributions from leaders in their respective fields along with abundant references This volume is dedicated to the subject of host microbe interactions Provides the latest research on host microbe interactions including new discoveries approaches and ideas Contains contributions from leading authorities on topics relating to molecular biology Informs and updates on all the latest developments in the field *Microbial Diversity in the*

Genomic Era Surajit Das,Hirak Ranjan Dash,2024-03-23 Microbial Diversity in the Genomic Era Second Edition presents techniques used for microbial taxonomy and phylogeny along with their applications and respective strengths and challenges Though many advanced techniques for the identification of unknown bacterium are available in the genomic era a far fewer number of the total microbial species have been discovered and identified to date With that in mind this book incorporates recently developed biosystematics methods and approaches to assess microbial taxonomy with suitable recommendations for where to apply them across the range of bacterial identification and infectious disease research Here international researchers in the field first provide a broad overview of microbial genomics research and microbiome directed medicine followed by sections on molecular tools for microbial diversity research extremophilic microbial diversity functional microbial diversity across application areas microbial diversity and infectious disease research and future directions for research Step by step methodologies are provided for key techniques along with applied case studies breaking down recent research studies into the practical components illuminating pathways for new studies across the field This new edition has been fully updated to address advances in the field of microbiome directed medicine and whole genome sequencing for studying microbial diversity considering both recent technological advances and new applications areas from extremophile studies to the latest approaches in human microbiome analysis Instructs in techniques used for microbial taxonomy and phylogeny with discussions of their applications and respective pros and cons Reviews the evolving field of microbial typing and the genomic technologies that enable comparative metagenomic analysis of complex microbial environments Covers microbiome directed translational research as well as whole genome sequencing for studying microbial diversity with newly added research protocols and case studies Reviews future applications in the field of microbiome directed medicine Features chapter contributions from global experts in the field

Molecular Interactions Between Bacterial Pathogens and Plants: Selected Contributions to the 14th International Conference on Plant Pathogenic Bacteria (14th ICPPB) Chiaraluce Moretti,Roberto Buonauro,Guido Sessa,Vittoria Catara,Michelle Teresa Hulin,George W. Sundin,2024-01-10 This Research Topic is dedicated In Memoriam of Dr Nicola Sante Iacobellis who contributed to the conception of this article collection This Research Topic collects the selected contributions to the 14th International Conference on Plant Pathogenic Bacteria 14th ICPPB The Impact of Plant Pathogenic Bacteria on Global Plant Health which was held in Assisi Italy from July 3 to 8 2022 Occurrence of bacterial disease in plant is the result of complex interaction between host bacterium and environment The mechanisms by which bacteria cause shifts in the biochemical and physiological processes required for the plant life cycle as well as the mechanisms by which hosts prevent or respond or defend against attack by bacteria are the central themes of this Research Topic

Bacterial Resistance Via Multidrug Efflux Pumps Robert Schulz,2011 Multidrug resistance against antibiotics is a common problem of treating bacterial infections One of the major contributors are efflux pumps that reduce the periplasmic concentration of antibiotics and other toxic compounds by pumping them out into the extracellular space This thesis

comprises several computational approaches to investigate the functions of the individual proteins of Escherichia coli's major efflux pump AcrAB-TolC. In the first part, the opening of the periplasmic tip of TolC, an outer membrane channel, was investigated by mutating specific residues in this region as it was shown in X-ray crystal structures. Upon docking to one of several possible inner membrane transporters, TolC is supposed to open, likely. In the present simulations, this opening was accomplished to a similar extent as in experiments. In the second part, the dynamics of AcrB's pumping process have been investigated by mimicking conformational changes which have been proposed by X-ray crystallographers. The outcome has been described by using a drug molecule as an unbiased measure. During this investigation, the binding pocket has been found to shrink peristaltically, which forced the drug to leave the binding pocket. In a subsequent work, the influence of its environment has been examined. Especially, the interaction with the surrounding water and amino acids revealed several essential features of the extrusion by AcrB. One interesting aspect was the stream of water which slightly assisted the extrusion of the drug and also shielded the electrostatic interaction of the protein and the substrate. This disfavored a strong binding of the drug to the amino acids of the exit channel. Furthermore, the inter- and intramonomeric interactions have been examined, and crucial conformational changes of the transition along the cycle have been highlighted. The effect of one particular mutation on the extrusion has been investigated in an additional work. The information presented in this thesis enhances the atomistic and functional understanding of E. coli's major efflux pump. By combining these data with experimental results, the mechanism of extrusion can be interpreted more clearly, which might also improve the chances to develop new antibiotics and inhibitors with a higher efficacy in the future.

Medicinal Plants and Antimicrobial Therapies Vinay Kumar, Varsha Shriram, Abhijit Dey, 2024-01-04 This book serves as an excellent comprehensive material covering the current understandings and updates on antimicrobial resistance (AMR) and the use of medicinal plant resources for tackling it. Chapters cover important aspects of AMR and strategies to address this threatening issue with medicinal plants and their resources. One health perspectives for addressing AMR have been presented for the readers. Antimicrobial medicinal plant therapeutic resources including crude extracts, active fractions, pure molecules, and essential oils, besides using them as functionalizing agents for nano-antimicrobials, have been covered. Further, plant endophytes as a source of antimicrobial compounds have been discussed. Chapters cover both the bactericidal as well as resistance reversal or potentiating combinatorial therapies, potencies of medicinal plant resources. The book also focuses on how medicinal plant resources effectively target major determinants of AMR. Use of metabolomics in understanding and targeting AMR mechanisms and identifying potent phytotherapeutics has been discussed as well. The book is a valuable read for both experienced and younger researchers working in the field of AMR and related fields in biomedicine, pharmacy, and clinical research.

The multiple roles of antibiotics and antibiotic resistance in nature Fiona Walsh, 2015-05-15 Antibiotics and antibiotic resistance have most commonly been viewed in the context of human use and effects. However, both have co-existed

in nature for millennia Recently the roles of antibiotics and antibiotic resistance genes have started to be discussed in terms of functions other than bacterial inhibition and protection This special topic will focus on both the traditional role of antibiotics as warfare mechanisms and their alternative roles and uses within nature such as antibiotics as signals or communication mechanisms antibiotic selection at low concentrations the non specific role of resistance mechanisms in nature e g efflux pumps evolution of antibiotic resistance and the role of persisters in natural antibiotic resistance *Bad Bugs in the XXIst Century: Resistance Mediated by Multi-Drug Efflux Pumps in Gram-Negative Bacteria* Attilio Vittorio Vargiu,Klaas Martinus Pos,Keith Poole,Hiroshi Nikaido,2016-08-12 The discovery of antibiotics represented a key milestone in the history of medicine However with the rise of these life saving drugs came the awareness that bacteria deploy defence mechanisms to resist these antibiotics and they are good at it Today we appear at a crossroads between discovery of new potent drugs and omni resistant superbugs Moreover the misuse of antibiotics in different industries has increased the rate of resistance development by providing permanent selective pressure and subsequently enrichment of multidrug resistant pathogens As a result antimicrobial resistance has now become an urgent threat to public health worldwide <http://www.who.int/drugresistance/documents/surveillancereport/en> The development of multidrug resistance MDR in an increasing number of pathogens including *Pseudomonas* *Acinetobacter* *Klebsiella* *Salmonella* *Burkholderia* and other Gram negative bacteria is a most severe issue Membrane efflux pump complexes of the Resistance Nodulation cell Division RND superfamily play a key role in the development of MDR in these bacteria RND pumps together with other transporters contribute to intrinsic and acquired resistance to most if not all of the antimicrobial compounds available in our drug arsenal Given the enormous drug polyspecificity of MDR efflux pumps studies on their mechanism of action are extremely challenging and this has negatively impacted both the development of new antibiotics that are able to evade these efflux pumps as well as the design of pump inhibitors The collection of articles in this eBook published as a Research Topic in Frontiers in Microbiology section of Antimicrobials Resistance and Chemotherapy aims to update the reader about the latest advances on the structure and function of RND efflux transporters their roles in the overall multidrug resistance phenotype of Gram negative pathogens and on strategies to inhibit their activities A deeper understanding of the mechanisms by which RND efflux pumps alone or synergistically with other efflux pumps are able to limit the concentration of antimicrobial compounds inside the bacterial cell may pave the way for new more directed inhibitor and antibiotic design to ultimately overcome antimicrobial resistance by Gram negatives *Bacterial Mechanisms of Antibiotic Resistance: A Structural Perspective* Vassiliy Bavro,Graeme L. Conn,Christopher Davies,2019-11-26 **Alternative Therapeutics Against Antimicrobial-Resistant Pathogens** Rebecca Thombre,Kamlesh Jangid,Ravi Shukla,Noton Kumar Dutta,2019-12-19 **Bacterial Resistance to Antimicrobials** Richard G. Wax,Kim Lewis,Abigail A. Salyers,Harry Taber,2007-12-05 The enormous genetic flexibility of bacteria jeopardizes the usefulness of currently available antibiotics and requires new approaches to antibiotic discovery and development

Antimicrobial resistance can be acquired in a short time frame both by genetic mutation and by direct transfer of resistance genes across genus and species boundaries Unde Bacterial Multidrug Exporters Akihito Yamaguchi, Kunihiro Nishino, 2018-08-30 This detailed volume utilizes our current understanding of the structural basis of multidrug recognition and multidrug efflux mechanisms to provide protocols involving these vital intrinsic membrane proteins widely distributed in bacteria Beginning with protocols for the structural analysis of bacterial multidrug exporters the book continues with sections on biochemical and bioengineering analysis computational analysis biomedical approaches as well as advanced technologies expected to be applied to multidrug efflux transport studies Written in the highly successful Methods in Molecular Biology series format chapters include introductions to their respective chapters lists of the necessary materials and reagents step by step readily reproducible laboratory protocols and tips on troubleshooting and avoiding known pitfalls Authoritative and practical **Bacterial Multidrug Exporters Methods and Protocols** serves as an ideal guide to this fast moving field of study **Microbiologia** ,1997 **Applied and Environmental Microbiology** ,2007 **Structure and Function of Multidrug Efflux Systems in Bacterial Antibiotic Resistance** Ruth Wong, 2007 Memoirs of the Institute of Scientific and Industrial Research, Osaka University Ōsaka Daigaku. Sangyō Kagaku Kenkyūjo, 2014 **Antibiotic and Biocide Resistance in Bacteria** Peter Gilbert, P. Gilbert, J.-Y. Maillard, A. F. Godfree, 2002 **Bacterial Efflux Systems** ,2025-11-28 Bacterial Efflux Systems Volume 722 covers experimental and computational approaches to study the function structure and mechanism of bacterial efflux pump systems The methods covered include preparation of samples for structural and functional analyses and in vivo activity analysis Specific chapters in this release cover Efflux pump functional assays NMR and functional analysis efflux pumps Methods for saturation mutagenesis and functional characterization Cooperation and functional interplay between pumps Mapping interactions HDX MS and much more Other sections cover Recombinant Expression and purification Reconstitution of a RND efflux pump in different artificial membranes for a structural biology approach Reconstitution of rnd and mfs efflux pumps for structural biology CusCBA system Expression and purification of efflux pumps of Gram positives Expression and purification of efflux pumps of archaea ABC transporters BmrA preparation for NMR and PatA PatB in peptidisc In situ structural analysis of efflux assemblies by cryoET Molecular dynamics simulations and so much more Includes interdisciplinary approaches for analysis of structure mechanism of the complex nanomachines Provides approaches to study function in vivo Presents methods for the reconstitution of nanomachines and for measuring activity

Whispering the Strategies of Language: An Emotional Quest through **Microbial Multidrug Efflux**

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