BBA - Biomembranes 1860 (2018) 868–877

Contents lists available at ScienceDirect

BBA - Biomembranes

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

Selective substrate uptake: The role of ATP-binding cassette (ABC) importers in pathogenesis

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ARTICLE INFO

Keywords:
ABC transporters
Pathogenesis
Metal transport
Peptide transport
Amino acid transport
Emerging therapy

ABSTRACT

The uptake of nutrients, including metals, amino acids and peptides are required for many biological processes. Pathogenic bacteria scavenge these essential nutrients from microenvironments to survive within the host. Pathogens must utilize a myriad of mechanisms to acquire these essential nutrients from the host while mediating the effects of toxicity. Bacteria utilize several transport proteins, including ATP-binding cassette (ABC) transporters to import and expel substrates. ABC transporters, conserved across all organisms, are powered by the energy from ATP to move substrates across cellular membranes. In this review, we will focus on nutrient uptake, the role of ABC importers at the host–pathogen interface, and explore emerging therapies to combat pathogenesis. This article is part of a Special Issue entitled: Beyond the Structure-Function Horizon of Membrane Proteins edited by Ute Hellmich, Rupak Doshi and Benjamin McIlwain.

1. Introduction

Bacterial colonization is dependent on the ability of the pathogen to obtain essential nutrients from surrounding environments. Bacteria have evolved to utilize a variety of strategies to acquire the necessary nutrients for homeostasis and pathogenesis. To resist bacterial colonization, a host can increase the antibacterial agents at the site of infection and/or limit the availability of nutrients to bacteria [1]. Bacteria respond to host-mediated nutrient deprivation, antibacterial defenses and other stressors, often taking advantage of changes in the host microenvironment associated with disease manifestation, by adapting strategies to overcome the host's immune system. Understanding how bacteria 'sense' and 'respond' to changes in host microenvironments will increase our understanding of the virulence factors that contribute to the pathogen's overall fitness or ability to cause disease. Bacterial transport proteins are necessary for nutrient uptake and are a major factor in host-pathogen interactions. Mutagenesis studies that target the components of ATP-binding cassette (ABC) transporters have revealed a subset of these transporters play a significant role in the survival and proliferation of pathogens within the host. This review summarizes research on a range of ABC importers with a role in virulence and highlights some of the ways researchers are targeting transport proteins to decrease pathogenicity.

1.1. ABC transporters: structure and function

A diverse group of transport proteins maintain a delicate balance of transport activities across cellular membranes. One family of transport proteins, ABC transporters, which consists of both exporters and importers, are conserved from bacteria to humans. Expression and transport activity of ABC transporters are tightly regulated to balance the need for essential nutrients and the effects of substrate toxicity. ABC exporters are responsible for the transport of diverse substrates such as antibiotics, lipids and proteins. ABC importers transport specific substrates across the membrane into the cytoplasm of bacteria and archaea [2]. ABC importers transport a broad range of substrates including sugars, metals, peptides, amino acids, and other metabolites [3]. Exporters and importers share the same mechanism of ATP binding and hydrolysis to power the translocation of substrates across the membrane [4].

ABC importers are further divided into three categories; Type I, Type II and Type III transporters (also known as energy-coupling factor (ECF) transporters) [5]. This distinction evolved out of the differences in overall architecture and variations in the transport mechanism of each subtype. Type I and II ABC importers utilize a substrate binding protein (SBP) to deliver substrate to the transporter. The SBP is located in the periplasm of gram-negative bacteria and is tethered to the cytoplasmic membrane or transporter in gram-positive bacteria [6]. These

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http://dx.doi.org/10.1016/j.bbamem.2017.08.011
Received 1 June 2017; Received in revised form 11 August 2017; Accepted 16 August 2017
Available online 25 August 2017

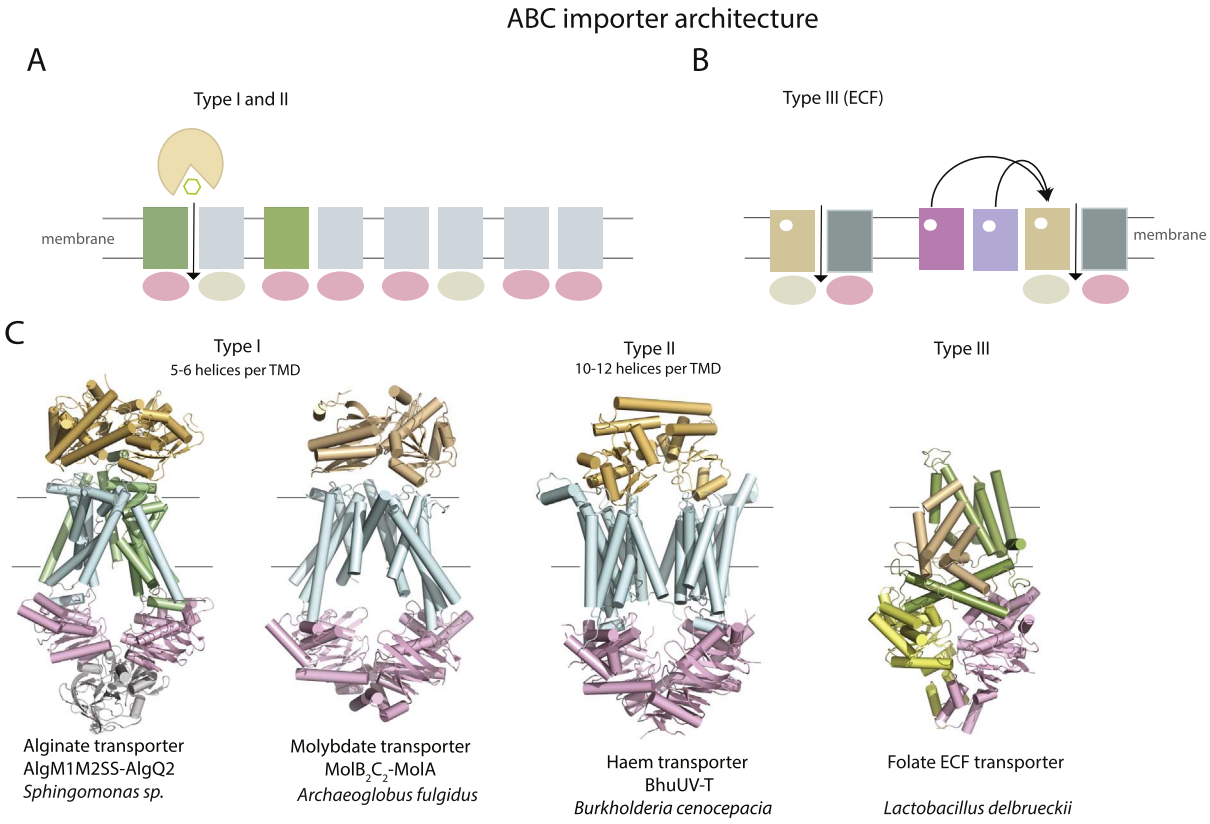

Fig. 1. Protein architecture and assembly demonstrates diversity of ABC transporters. (A) ABC transporter assembly for Type I and II importers. The transmembrane domain (TMD), nucleotide binding domain (NBD) and substrate binding protein (SBP) are represented by ovals, rectangles and spheres with an opening for substrate binding, respectively. Importers consist of homo- and heterodimers of TMD and NBD components. (B) For Type III ABC transporters, each transporter is comprised of the energizing module and (EcfT, EcfA, EcfA′) and EcfS or multiple EcfS components share an energizing module. (C) Four representative examples of ABC transporters based on the Type I, Type II and Type III classifications. Type I Alginate transporter AlgM1M2S_2-AlgQ2 from *Sphingomonas sp.* (PDB ID: 4TQU) and Molybdate transporter MolB_2C_2-ModA from *Archaeoglobus fulgidus* (PDB ID: 2ONK). Type II heme transporter BhuU_2V_2 in complex with SBP, BhuT, from *B. cenocepacia* (PDB ID: 5B58). The TMDs are colored in light cyan or light green, NBDs in light yellow or light pink and the SBPs are colored light orange. Accessory domains in Type I transporters are colored grey. For the Type III transporter, folate ECF transporter from *Lactobacillus delbrueckii*, the NBD components are colored in light pink and yellow, the S-component in wheat and the T-component in green.

SBPs recognize and deliver substrates to the cognate ABC transporter. The SBPs are categorized into several clusters based on overall structure of the proteins [7]. Despite architectural differences, all SBPs contain at least two domains or lobes with a pocket at the interface for substrate binding.

Type I and II transporters consist of transmembrane domains (TMD) embedded in the lipid bilayer that form the translocation channel and nucleotide-binding domains (NBD) for hydrolyzing ATP. While there are several conserved motifs in the NBDs that play a role in ATP binding, sequence conservation amongst TMDs is low. However, the overall topology between types of ABC transporters seems to be conserved; Type I ABC transporters typically have 5–6 helices per TMD while Type II has 10–12 helices. TMDs and NBDs dimerize and assemble the minimal unit of an importer, with the SBP as the fifth component of the complex (Fig. 1A) [2]. Some importers also contain an accessory domain as part of the NBD, often conferring the ability to regulate transport activity [8]. Different from Type I and II importers, ECF transporters consist of four components, which include the substrate-specific binding component (EcfS), transmembrane component (EcfT) and two nucleotide-binding domain components (EcfA and EcfA′). EcfT, EcfA and EcfA′ are the conserved components which make up the energizing module, while EcfS is substrate specific (Fig. 1B) [9]. The EcfS is embedded in the lipid membrane and binds to substrates, replacing the need for the SBPs present in Type I and II importers [10]. The general architecture and assembly of ABC transporters are depicted in Fig. 1.

In addition to the unique architecture, the mechanism of transport differs between the classes of importers. While there are variations in the details of the mechanisms of Type I and II transporters, in general, the TMDs rearrange providing alternating access from the one side of the bilayer to the other, allowing for unidirectional transport. Structural studies of ABC transporters in complex with SBPs have revealed how each SBP forms a complex with the TMDs of its cognate transporter to deliver substrate to the translocation pathway. Binding of substrate by the SBP and formation of the transporter complex determines selectivity for substrate transport (for comprehensive reviews on the mechanism and selectivity of ABC transporters see [5,11–13]). Alternatively, the S component of the ECF transporter binds substrate and rearranges position to deliver it across the lipid bilayer [14]. In a mechanism unique to Type III transporters, a subclass of ECF transporters bind a number of EcfS components interchangeably [10]. These transporters are composed of the same EcfT and EcfA and EcfA′ modules, but utilize different EcfS components depending on which substrate is transported (Fig. 1B) [15].

1.2. ABC transporters: nutrient acquisition and pathogen virulence

Nutrient acquisition is essential for all bacteria, commensals and pathogens, to establish colonization in the host. Many importers play a crucial role in nutrient delivery; for example, ECF transporters for riboflavin are commonly found in the *Listeria monocytogenes*, *Bacillus subtilis* and *Clostridium difficile* while the Type I zinc importer, ZnuABC is present in *Brucella abortus*, *Yersinia pestis*, *and Proteus mirabilis* [16,17]. Pathogenic bacteria can rapidly adapt to changing host