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

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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.

Keywords

ABC transporters; Pathogenesis; Metal transport; Peptide transport; Amino acid transport; Emerging therapy

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

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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 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 microenvironments, through nutrient acquisition by select ABC transporters. The mechanisms of nutrient acquisition are key virulence determinants used by pathogens to mediate disease [16,17]. There are many essential nutrients bacteria need for survival, but in many cases there remains a lack of information linking these systems to pathogen virulence, primarily assessed by phenotypic characterization of transporter mutants in animal models of infectious diseases. While many key transport systems remain to be identified, a few select ABC importers have been shown to be critical to the virulence of bacteria, establishing these proteins as virulence factors (Table 1). These virulence factors transport an array of substrates including transition metals, peptides and amino acids. In this review, we take a closer look at ABC importers as virulence determinants of pathogenic bacteria, often supported by loss of virulence through genetic deletion of components of essential ABC transporter genes.

1.3. Metal transporters

All bacteria require essential metals to carry out biological processes. Iron, cobalt, nickel, copper, zinc, and manganese are all trace elements that play a role in a myriad of biological processes, including enzyme cofactors, necessary structural components of proteins, or resistance to oxidative stress [18]. To fulfill this need for trace metals, bacteria utilize several metal transport systems that tightly regulate the uptake of metals, allow for expulsion of excess metals to avoid toxicity, and maintain homeostasis. To defend against the invasion

of bacteria, mammals have developed ways to restrict the availability of essential metals in the host environment, a process called *nutritional immunity* [19,20]. For example, to limit a pathogen's access to iron, the host sequesters nutrients *via* lactoferrin or transferrin bound iron and hemopexin in complex with heme [21]. Bacteria have adapted to iron sequestration by utilizing siderophores and receptors that can bind these host-derived proteins for iron acquisition [22,23]. Once acquired, iron and heme substrates are transported into the cytoplasm by a series of transport proteins, such as the TonB-dependent system, symporters, and ABC importers. While the type of transport proteins that are involved in pathogenicity is broad and covered in many comprehensive reviews [19,24,25], we have selected a few metal ABC importers to highlight the role they play in maintaining bacterial virulence.

Zinc is required for the proper folding and stability of proteins in the cell and serves as a catalyst for many enzymes. Even though zinc is an essential nutrient, it also plays a role in the host defense mechanism as high levels of zinc can be toxic to a bacterium [25]. In addition to other transport proteins, ZnuABC, a high affinity zinc MZT (Manganese/Zinc/Iron Chelate Uptake) family transporter, contributes to pathogen survival in the infected host. *znuA*, *znuB* and *znuC* encode for the substrate binding protein, the transmembrane domain and the nucleotide-binding domain, respectively. In *B. abortus*, an intracellular pathogen capable of infecting domestic animals and occasionally humans, *znuA* is required for growth in zinc-limited conditions. Loss of ZnuA decreased the ability of *B. abortus* to replicate in RAW 264.7 macrophages and increased clearance in BALB/c mice [26]. In *Salmonella enterica* serovar Typhimurium, a *znuA* mutant strain was also less virulent in BALB/c and DBA-2 mice compared to the wildtype strain [27]. These data correlate with a previous study of a *S.* Typhimurium *zur* (*znu* regulatory gene) and *znuC* knockout [28]. A *znuA* mutant knockout in *Campylobacter jejuni* shows that the SBP is essential for colonization of the chick gastrointestinal tract [29]. For *Moraxella catarrhalis*, an otitis media (OM) pathogen associated with clinical exacerbation of chronic obstructive pulmonary disease (COPD), *znuABC* is necessary for invasion of human adenocarcinoma epithelial A549 cells and persistence in respiratory tract of BALB/c mice [30].

While *znu* mutations resulted in a marked decrease in virulence in some pathogens, others showed no phenotype with loss of the *znu* system. However, bacterial attenuation was observed when *znu* deletions were combined with other mutations or when mutant strains were coinfected with wildtype strains. In uropathogenic *E. coli* (UPEC), loss of ZnuA and ZupT (ZRT-, IRT-like Protein family) proteins decreased the bacterial load in the murine urinary tract and kidneys in both a single-strain infection and when the mutant was coinfected with the wild type strain [31]. In *Y. pestis*, deletion of *irp2*, the Ybt siderophore synthetase, in the *znuABC* mutant background resulted in a significant loss of virulence due to a defect in zinc acquisition [32,33]. While the *znuABC* mutant has a severe *in vitro* growth defect, the *irp2 znuABC* mutant is attenuated in the septicemic plague mouse model [32]. Further, zinc uptake in *Y. pestis* is a concerted effort between components of the Ybt system, ZnuABC, and possibly a second high-affinity zinc transporter, yet to be identified, which all contribute to virulence [33,34]. During coinfections of *Acinetobacter baumannii*, an opportunistic and nosocomial pathogen, the loss of the TMD strain results in wildtype outcompeting the *znuB* mutant for colonization in lungs and livers of C57BL/6 mice [35]. In *P. mirabilis*, a common cause of complications in urinary tract infections, the NBD *znuC*

mutant strain failed to survive in the bladder and kidneys of CBA/J mice when coinfected with wildtype, indicating that ZnuABC contributes to the fitness of *P. mirabilis* in murine urinary tract infections [36]. Co-challenge studies show that ZnuABC is not required to colonize the host, but offers a competitive advantage during infections.

The ability to transport manganese into the cell is critical for the detoxification of free radicals and protection against oxidative damage caused by hydrogen peroxide. SitABCD, YfeABCD, PsaABC, and MntABC have all been implicated in manganese transport, and their role in divalent cation transport also places them in the MZT family [37,38]. These importers play a role in virulence in *S.* Typhimurium and *Neisseria gonorrhoea* [39]. In *N. gonorrhoeae*, a mucosal pathogen often associated with the genitourinary tract, MntABC is the Mn^{2+} and Zn^{2+} transporter. MntC, the SBP, recognizes both substrates with the same binding pocket and has a similar affinity to each substrate, approximately 1 μM [40]. Individual mutations of *mntAB* and *mntC* show a significant reduction in invasion of primary cervical epithelial cells, and both mutants are deficient in forming biofilms [40]. In *Bartonella henselae*, a bacterium commonly transmitted to humans through a cat flea or cat bite, deletion of the SBP and NBD, *sitA* and *sitB*, decreases the ability for the pathogen to survive in cat fleas. Interestingly, the *B. henselae sitAB* knockout does not impact the invasion of human endothelial cells, but has a decreased rate of survival when compared to the control [41].

Iron, in its many forms, is required for essential processes in the bacteria, and iron deficiency can impact nucleotide synthesis, ATP production, and the activity of numerous critical enzymes [42,43]. SitABCD along with other transport proteins, MntH (NRAMP family) and Feo (GTPase), import ferrous iron. In avian pathogenic *E. coli*, APEC O78 strain $_{x}$7122, *sitABCD* mutant strain was partially attenuated in its ability to colonize the lungs, livers and spleens of infected chickens and was further attenuated in a coinfection model with wildtype. Loss of both divalent cation transporters, *sitABCD mntH* demonstrated the greatest reduction in bacterial load in the lungs, liver and spleen. Interestingly, the bacterial load of the *sitABCD feo* mutant was lower in the lungs and spleen, but not the liver, suggesting tissue tropism [44]. In contrast, MntH and Feo single and double mutants did not reduce bacterial load, suggesting an important role for SitABCD in avian pathogenic *E. coli* colonization. Additionally, *S.* Typhimurium requires both divalent cation transporters, MntH and SitABCD, for full virulence in wildtype $Nramp^{G169}$ mice following intraperitoneal infection, with only partial attenuation for each individual mutation [45]. Single mutations of *Y. pestis yfeAB* or *mntH* did not show a significant loss in virulence. However, the *yfeAB mntH* double mutant shows a substantial decrease of virulence in the bubonic plague mouse model compared to the parent strain. Furthermore, double mutants were fully avirulent in a mouse model of pneumonic plague [33]. It is interesting to note that a *feoB yfeAB* double mutant in *Y. pestis* also results in a decrease of virulence in the bubonic plague mouse model when compared to the parent strain, whereas single mutations did not [46].

For nickel and copper, studies indicate the ability to properly regulate the transport of these metals is important for the survival of bacterial pathogens [1]. Copper is a common cofactor for oxidases and plays a role in the formation of reactive oxygen species (ROS)

[25]. However, due to copper toxicity and antibacterial properties [47,48], bacteria utilize a number of mechanisms, including copper exporters, to transport copper out of the cytoplasm where it is detoxified or released into the extracellular space [1,49,50]. Nickel is a common cofactor of many enzymes, including urease, an enzyme that hydrolyzes urea into ammonia and carbamate, and is important for maintaining a neutral cytosolic pH [51]. Nickel uptake has been shown to be essential for colonization of pathogens, including *Staphylococcus aureus* and the human gastric pathogen *Helicobacter pylori* [52]. Nickel import is controlled by several uptake systems, including a member of the Peptide, Opine and Nickel Uptake (PepT) transporter family, CntABCDF, and a recently discovered nickel transporter, NiuBDE. Recent work has identified gastric *Helicobacter* species that have acquired nickel transporter genes through horizontal gene transfer [53]. Whereas NiuBDE and NixA function independently to transport nickel, both transporters participate in nickel-dependent urease activation at pH 5 and promote survival of *H. pylori* in the acidic environment of the stomach. However, only NiuBDE transports transport nickel for urease activation at neutral pH and is essential for colonization of the mouse stomach suggesting preferential transport of nickel with changing microenvironments [53]. *S. aureus cntABCDF*, originally annotated as *opp1*, is a cobalt and nickel transporter that impacts urease activity and bacterial colonization in the systemic and urinary tract infection models. BALB/c mice infected with the *cnt* operon deletion strain were twice as likely to survive compared to infection with wildtype, and in the urinary tract infection mouse model, bacterial load of kidneys and bladders was lower for the *cnt* mutant strain [54].

1.4. Amino acid transporters

With limited resources, pathogenic bacteria, such as *S. aureus* and *Salmonella*, rely on numerous host amino acids and require multiple amino acid transporters to fulfill their metabolic requirements for growth, persistence, and virulence [55,56]. Since amino acids are essential nutrient sources for carbon and nitrogen, as well as, recycling amino acids for protein synthesis in bacteria, they are central to the host–pathogen metabolic interaction. Amino acids also play a role in cellular responses of pathogens. Increased levels of asparagine in the host induce expression of bacterial virulence genes during *Streptococcus pyogenes* infection [57]. *Francisella tularensis* and *Mycobacterium tuberculosis* require *de novo* tryptophan synthesis to circumvent the depletion of host tryptophan as a result of the active T cell response [57]. *Chlamydia trachomatis* evades host nitric oxide production by limiting arginine, which deprives nitric oxide radicals (iNOS) of substrate [58]. For euedaphic human pathogenic bacteria, such as *Bacillus anthracis* and *L. monocytogenes*, ammonia is the primary nitrogen source in the non-host environment [59]; in contrast, L-glutamine and L-glutamate are widely available nitrogen source in the host environment [60]. Additionally, the host increases L-glutamine levels to activate immune defenses, which is necessary to maintain function of lymphocytes and macrophages [61]. Nutrient uptake by amino acids transporters is important for bacterial survival, and many of these transporters have been identified as virulence factors utilized by pathogens during successful infection.

L-glutamate, an essential amino acid for bacterial growth and intermediate product for ammonium assimilation, is a precursor for the synthesis of the antioxidant glutathione [62,63]. *Neisseria meningitides*, an exclusively human pathogen, requires glutamate for

growth *in vitro* and the intracellular milieu [64]. Specifically, the SBP GltM (*NMB1964*) and the TMD GltT (*NMB1965*) of the meningococcal L-glutamate ABC transporter, a homolog of phospholipid-uptake MlaBDEF transporter in *E. coli*, imports glutamate under low Na^+ conditions [65,66]. This transporter is essential for survival during infection of epithelial cells and resistance to neutrophil oxidative burst [66–68]. The null *N. meningitidis ΔgltT ΔgltM* strain was defective in the internalization into human umbilical vein endothelial cells and the human adenocarcinoma epithelial A549 cells [69]. A recent study showed GltT-GltM is necessary for glutamate uptake, which is used to produce glutathione, for increased meningococcal survival during infection of human brain microvascular endothelial cells [70].

Like glutamate, L-glutamine also serves as the primary nitrogen source of pathogenic bacteria [71]. The glutamine GlnHPQ transporter is a member of the Polar Amino Acid Uptake Transporter (PAAT) family. GlnH, the SBP, is found in the periplasm in gram-negative bacteria, and *E. coli* GlnH has an affinity of 0.3 μM for glutamine [72]. GlnHPQ in combination with glutamine synthetase, glnA, is required for virulence of *S.* Typhimurium. Double mutants of the glutamine synthetase and the SBP or the NBD, *ΔglnA ΔglnH* or *ΔglnA ΔglnQ*, resulted in a 10^4-fold decrease in competitive growth of the mutant strains and attenuated growth in mice compared to the wildtype strain [73]. The glutamine ABC transporter is required for virulence of Group B streptococci. Group B streptococci can cause sepsis in newborns and adults with chronic conditions, such as, diabetes or liver disease and *Streptococcus pneumoniae*, an opportunistic pathogen in the human respiratory tract, causes ear and sinus infections, pneumonia, and meningitis [74]. In gram-positive *S. pneumoniae*, deletion mutants of the fused TMD and SBP GlnP_H and the NBD GlnQ showed significant attenuation in the ability to cause pneumonia and septicemia in a mouse infection model [75]. When the macrophage-like cell line J774A.1 was infected with *gln* mutants, the number of internalized and viable pneumococci was significantly lower than wildtype. These phagocytosis studies suggest the Gln uptake system might play a role in resistance to oxidative stress during host infection [75]. In Group B streptococci, a *ΔglnQ* strain shows decreased fibronectin adherence, lower invasion of human adenocarcinoma epithelial A549 cells *in vitro*, and decreased virulence in neonatal rats [76]. Inactivation of *L. monocytogenes glnPQ* abolished glutamine uptake, lowered the response of type I interferon in infected bone marrow-derived macrophages, and down-regulated transcription of virulence factors, such as, *hly*, *plcA*, *plcB*, and *actA* [77]. Liver and spleen colonization of C57BL/6 mice intravenously injected with the *ΔglnPQ* strain was reduced compared to the wildtype strain, leading to a 30-fold and a 10-fold decrease in bacterial load of the liver and spleen, respectively [77].

As precursors for membrane fatty acids, BCAAs are key co-regulators of growth and virulence of pathogenic bacteria. Interestingly, the *livHMGF* BCAA transporter, a member of the Hydrophobic Amino Acid Uptake Transporter (HAAT) family, has two SBPs. The leucine/isoleucine/valine (LIV) SBP, also known as LivJ, has been co-crystallized bound to isoleucine, leucine and valine with dissociation constants of 0.4, 0.4, and 0.7 μM, respectively [78,79]. Additionally, LivJ weakly interacts with alanine, serine, threonine and phenylalanine [80,81]. LivK, known as the leucine-specific (LS) SBP, recognizes leucine with a binding affinity of 0.4 μM and surprisingly, phenylalanine with an affinity of 0.18

μM [82]. The structure of LivK bound to leucine and phenalanine identified residues in the binding pocket responsible for determining substrate specificity for the bulkier hydrophobic amino acids in comparison to LivJ [83]. Interestingly, the *ΔlivHMGF* strain has decreased virulence in disease models dependent upon serotype-specific phenotypes. Loss of *livHMGF* in *S. pneumoniae* serotype 4 strain TIGR4 leads to lower virulence in the pneumoniae mouse model, a modest decrease in virulence in the systemic model, which is not statistically significant, and no difference in the nasopharyngeal infection model. However, in the *S. pneumoniae* serotype 3 clinical isolate 0100993, the *ΔlivHMGF* strain is attenuated in the pneumoniae and systemic model [84].

The ability to bind and import amino acids is important for bacteria to establish infection. In many cases, loss of SBPs of amino acid uptake ABC transporters attenuates infection. A recent study demonstrated that deletion of several amino acid SBPs in *M. catarrhalis*, including the lysine and ornithine transporters, showed a reduced ability to invade human adenocarcinoma epithelial A549 cells [85]. In the gram-positive human pathogen, *L. monocytogenes*, the SBP of cysteine transporter, CtaP, was shown to contribute to virulence in a murine model of intravenous infection. The *ΔctaP* strain had increased membrane permeability and acid sensitivity, reduced bacterial adherence to host cells, and lower colonization of the gastrointestinal tract of mice [86].

Expression of DalS, the SBP of D-alanine ABC transporter (*STM1633–STM1636*) in *S.* Typhimurium, limits exposure to oxidative damage elicited by D-amino acid oxidase (DAO) in neutrophils. The *dalS* mutant was more susceptible to DAO-dependent killing during host infection [87]. In addition, the *ΔdalS* strain showed decreased replication in the RAW 264.7 macrophage cells compared to the wildtype strain. The mean survival time of C57BL/6 mice orally infected with wildtype *S.* Typhimurium was a day shorter than mice infected with the *dalS* mutant [88].

MetQ is the SBP of the methionine-uptake MetQNP ABC transporter in *S. pneumoniae*, and is another serotype-specific virulence factor [89]. Coinfection of wildtype and *ΔmetQ* strains of *S. pneumoniae* serotype 3 clinical isolate 0100993 showed attenuated colonization of the *metQ* mutant in the nasopharynx and spleens of CD1 mice [90]. However, for single-strain infections, virulence of the *metQ* mutant in CD1 mice was unchanged compared to wildtype in the *S. pneumoniae* serotype 3 strain [91]. In contrast, bioluminescent imaging in real-time showed a *metQ* mutation in *S. pneumoniae* serotype 2 strain D39 showed significantly attenuated virulence in an acute pneumonia mouse model [92]. Similarly, *metQ* of the methionine MetNIQ transporter plays no role in epithelial cell invasion of *M. catarrhalis*, but facilitates persistence in the murine lungs [85].

1.5. Peptide transporters

Small peptides are transported by the PepT sub-family of ABC importers. This subset is classified by structure and sequence identity and includes well-characterized peptide transporters, *dppABCDF*, *oppABCDF*, and *sapABCDF*. Interestingly, as mentioned above the PepT family also includes *nikABCDF* and *cntABCDF*, which are not known to import peptides, but share the overall structural similarity to the peptide transporters in this family [93]. Peptide transporters are necessary for the import of peptides derived from

host proteins, like hemoglobin and proteasomal degraded proteins [94,95] as a source of nutrients, and for importing environmental cues for cellular functions, such as, chemotaxis, conjugation, and sporulation [96–98]. The di-peptide transporter, *dppABCDF* [99], has been implicated in virulence, but only the *oppABCDF*, *sapABCDF and yejABEF* systems are directly identified as virulence factors [100–102].

In addition to their role in metabolic activity, the *sap* and *yej* systems recognize antimicrobial peptides (AMPs) produced by the host innate immune system. A wide range of organisms, including eukaryotes, plants, and bacteria, produce AMPs [103]. During infection by pathogenic bacteria, host epithelial cells up-regulate the expression of AMPs and recruit AMP-secreting cells to the infection site, such as macrophages or neutrophils. Peptide ABC transporters recognize small cationic AMPs, including α-defensins, β-defensins, cathelicidins, and polymyxins. The role of ABC transporters in peptide uptake and AMP resistance is vital for pathogen survival and replication in the hostile host environment.

Bacteria scavenge peptides from the environment, which provide a source of carbon, nitrogen, and/or amino acids, during homeostasis and pathogenesis. *Lactococcus lactis* OppA, the SBP of the *opp* system, binds peptides from 4aa up to 35aa long [104]. Structural and functional studies indicate that OppA from L. *lactis* can bind peptides of varying length 5, 8, 9, 12 and 20aa, with an affinity range from 0.1–100 μM, with the highest affinity for 9aa peptides [105]. *M. catarrhalis oppA* is necessary for invasion of human adenocarcinoma epithelial A549 cells [85]. Pulmonary clearance of the Δ*oppA* strain of *M. catarrhalis* was increased compared to the parent strain in the mouse model [106]. Deletion of *oppB*, the TMD, in *Bacillus thuringiensis*, which is closely related to the pathogen causing food-borne gastroenteritis *Bacillus cereus*, significantly reduced the mortality rate of *Galleria mellonella* insect larvae by *B. thruingiensis* spores [107]. The *opp* system is linked to the up-regulation of other virulence-associated genes, such as, *speB*, for cell adhesion and *plcR*, a virulence regulator [107,108]. However, the *opp*-transported peptide and the signal transduction pathway to induce virulence genes are still unknown.

The *sapABCDF* system is a key virulence factor of AMP resistance, *in vivo* colonization, and persistent survival in the host environment. The AMP resistance of the *sap* operon was first described in *S*. Typhimurium and *Dickeya dadantii* (previously known as, *Erwinia chrysanthemi*) [109,110]. Nontypeable *Haemophilus influenzae* (NTHi), the causative agent of middle ear infection, has increased *sap* promoter activity during colonization of the chinchilla model of otitis media [111]. SapA, the SBP, is thought to be specific for AMPs with positive charges, including cathelicidin, melittin, and defensins [112]. Although the structure of SapA bound to AMPs has not been solved, deletion of *sapA* increases sensitivity of NTHi to chinchilla β-defensin-1 [111]. Loss of *sapA* also leads to a decrease in human bronchial epithelial cell and chinchilla middle ear epithelial cell adhesion of NTHi [113]. Virulence of NTHi was attenuated in the chinchilla middle ear and nasopharynx by the Δ*sapA* strain [111]. Importantly, humans infected with a *sapA* mutant of *Haemophilus ducreyi*, a causative agent of the sexually transmitted infection that causes skin ulcers, formed pustules at half the rate of the parent strain [114]. The TMDs of the sap operon have also been proven to play a role in virulence in these pathogens. AMPs are translocated

across the cellular membrane *via* the TMDs. The permease-deficient Δ*sapBC* strain exposed to sub-lethal concentrations of human cathelicidin (LL-37) and human β-defensin-3 (hBD3) showed localization and accumulation of the AMPs in the periplasm and on the NTHi lipid membranes [115]. While the *sap* operon in *H. ducreyi* has not been linked to defensin resistance, *sapB* and *sapC* are necessary for LL-37 resistance and virulence in the host. The *sapBC* mutant had decreased pustule formation in human volunteers even at dose 10-fold higher that the parent strain, and resulted in the full attenuation of *H. ducreyi* virulence [116]. Additionally, *sapD* is associated with potassium homeostasis, and deletion of the NBD attenuated survival of NTHi in the chinchilla model [117].

YejABEF confers resistance to cationic and cyclic AMPs, such as melittin, protamine, polymyxin B, and β-defensins [118]. In murine macrophage cells, a microarray showed that *S*. Typhimurium *yejB*, *yejE* and *yejF* are induced at least 2-fold during infection [102]. Additionally, expression of *yej* genes increased when *Brucella melitensis*, which can be transmitted to humans by the stable fly from farm animals, was treated with polymyxin B [119]. Deletion of the TMD, *yejE*, or the entire operon, *yejABEF*, in *B. melitensis* display decreased ability to invade and replicate in activated macrophages. These mutants had a bacterial load four orders of magnitude lower than the parent strain by the end of the incubation period [119]. Loss of the NBD in *S.* Typhimurium Δ*yejF* also had attenuated replication in hBD3 and hBD4 expressing intestine 407 cells, hBD2 expressing HeLa cells, and hBD1 and hBD2 expressing Caco-2 cells [118]. The attenuated growth of *B. melitensis* and *S.* Typhimurium mutants in mouse Peyer's patches, liver and spleen demonstrate the role of the *yej* transporter in bacterial survival during infection [118,119]. While the structure and substrate selectivity of *yejA* is not as well characterized as other peptide SBPs, the *yej* system is essential for AMP resistance and bacterial virulence.

2. Discussion

There are many factors that influence bacterial survival and virulence within the host. As highlighted in this review, many bacteria colonize different tissues with a range of efficiencies, reflecting tissue tropism. Pathogens also encounter many assaults *in vivo* (*e.g.*, fluctuations in pH, oxidative stress, toxins, nutrient sequestration, microbiota); therefore, changing microenvironments may impact the efficiency to acquire necessary nutrients for pathogenesis. In a screen of knockout mutants of SBPs and ABC transporters, Murphy and colleagues showed different patterns of activity in differing assay systems, suggesting that different SBPs and ABC transporters function at different stages in the pathogenesis of infection [120]. Collectively, these results indicate that ABC transporters are nutritional virulence factors.

ABC transporters are pivotal for transport of numerous diverse and essential substrates necessary for virulence. For many of the transport systems covered in this review, loss of substrate delivery by the SBP affects microbial virulence. Recognition of the substrate by the SBP is the first step in substrate selectivity and is critical to microbial fitness in changing microenvironments. Likewise, disruption of substrate delivery to the TMD-NBD complex can affect virulence attributes from colonization and survival to mechanisms of pathogenesis dependent upon substrate uptake. Recent advances in our understanding of

substrate-SBP and SBP-TMD interactions, necessary for the delivery of substrate into the cell, have focused development of target therapeutics for these interactions to effect disease manifestation.

2.1. Targeting pathogenesis: advances and therapeutic approaches

Bacterial pathogens are a significant threat to human health, and the continued emergence of antimicrobial resistance has only amplified this danger. The mechanisms utilized by bacteria to acquire essential nutrients within the host and the nutrient sequestration of the immune response during infection can be capitalized upon to thwart the colonization of bacteria. Understanding the structure of ABC importers, defining the specificity of substrate translocation, and deciphering how these transporters fit into the overall survival mechanism of an organism has led to interesting approaches to combating these diseases.

2.2. Substrate mimics

ABC importers act as a natural gateway into the cell. Antibiotics that mimic transporter substrates can exploit these uptake pathways in a ‘Trojan horse’ mechanism. Bacteriocins are natural antibiotics produced by bacteria to give them a competitive advantage over their neighbors. These antibiotics mimic essential nutrients, such as iron-siderophore complexes or peptides, and are imported to the cell by ABC transporters [121]. A peptidyl nucleoside antibiotic, pacidamycin, is imported by the *nppA1A2BCD* system and inhibits translocase I, MraY, in *Pseudomonas aeruginosa* [122,123]. The *E. coli yejABEF* system imports microcin C, a peptide-nucleotide antibiotic, which blocks the synthesis of $tRNA^{asp}$ [124]. The SBP of *E. coli* an iron-siderophore transporter, FhuD, binds a siderophore-antibiotic conjugate that inhibits seryl-tRNA synthetase known as albomycin (Fig. 2B) [125]. ABC importers also play a role in the uptake of synthetic antibiotics. The dipeptide transporter, *dppABCDF*, imports orthine carbamoyltransferase inhibitor phaseolotoxin and translation initiation inhibitor kasugamycin [126,127]. Additionally, kasugamycin and translation termination inhibitor blasticidin are imported *via* the *oppABCDF* system in *E. coli* [127].

2.3. Inhibition of substrate binding

Inhibitors that specifically target the SBPs of ABC transporters have also proven to be successful drug candidates. *S.* Typhimurium ZnuABC is one on such example. Two zinc-binding compounds, RDS50 and RDS51, were shown to inhibit *S.* Typhimurium growth and decreased pathogen invasion of intestinal epithelial Caco-2 cells. The crystal structure of the RDS51-Zn(II)-ZnuA complex reveals that RDS51 (1-[(2-chlorophenyl)methyl]-4-phenyl-1H-pyrrol-3-hydroxamic acid) binds near the zinc-binding site of the SBP (Fig. 2). RDS51 bridges the globular domains by forming hydrophobic contacts with residues on both sides of the binding pocket and coordinates with the zinc ion, either disrupting ZnuABC complex formation or preventing the release of zinc [128].

2.4. Vaccine targets

Vaccines are an alternative approach to antibiotics, and production of antibodies against the SBPs has proven to be a promising therapeutic. SBP *oppA*-immunized mice challenged with *Y. pestis* had an increased survival time in comparison to the control group [129].

Additionally, mice immunized with OppA had a significant reduction of *M. catarrhalis* colonies in the pulmonary clearance model [130]. Further work is needed to elucidate the mechanism of *oppA* virulence and how OppA-raised antibodies protect against bacterial infection. A co-immunization study of *S. pneumoniae*, PiuA and PiaA, showed using the two iron SBPs antigens produced additive protection in mice [131]. Mechanistic studies explained how anti-PiaA and anti-PiuA polyclonal rabbit antibodies did not inhibit growth of *S. pneumoniae* in iron-restricted conditions, indicating these antibodies protect mice against *S. pneumoniae* infection by promoting opsonophagocytosis, not restricting iron transport by the SBPs [132]. Immunization with recombinant manganese SBP, MntC, elicits an anti-MntC IgG response capable of protecting mice against *S. aureus* [133]. Structural and functional studies revealed the mechanism for MntC-induced immune protection. The MntC monoclonal antibodies interact with each of the globular lobes of MntC, including the binding pocket, potentially blocking the SBP-TMD interface and manganese binding (Fig. 3) [134]. In addition, a *S. aureus* vaccine that incorporates multiple antigens that target different virulence mechanisms, including MntC, has been successful in clinical trials [135].

3. Conclusion

Although in its infancy, the use of ABC importers as potential targets to thwart pathogenic bacteria has proven to be promising for the development of new therapies. While the approaches differ from mimics to inhibitors, the most promising cases capitalize on substrate recognition of the SBP or complex formation between the SBP and transporter. In these cases, loss of substrate binding or selective transport of substrates results in an inability of the bacterium to acquire necessary nutrients to support pathogenesis. Targeting biological systems essential for nutrient acquisition provides a viable alternative to therapeutics directed at cell surface proteins, which may be prone to antigenic variability or phase variation. Ultimately, therapeutics that target ABC importer functions have the potential to disrupt the virulence pathways of bacteria [136]. As demonstrated by the studies mentioned above, targeting the SBP has identified potential therapeutics to restrict nutrient uptake by pathogens, thereby having the potential to decrease pathogen virulence. Recent studies of other transporters, including ArcB and TAP, have identified inhibitors that lock the conformation of transporter and prevent substrate translocation [137,138]. Further therapeutic studies could target the translocation pathway of TMD and ATP-hydrolysis of the NBD to prevent transport of nutrients and bacterial pathogenesis. Although more work is needed, current research reveals loss of function of certain ABC transporters results in the loss of virulence, increasing the promise of ABC importer targeted therapeutics by expanding the possible avenues to fight infections in the future.

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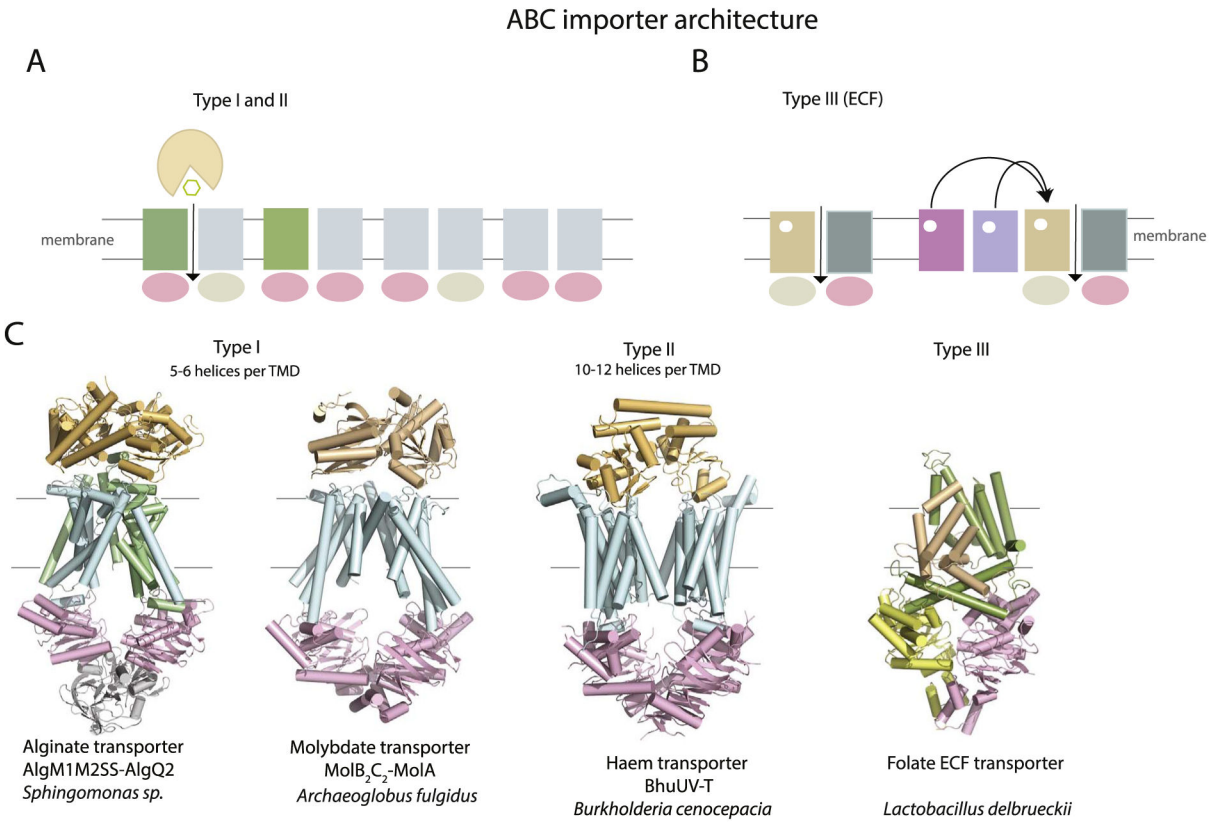


Fig. 1.

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.

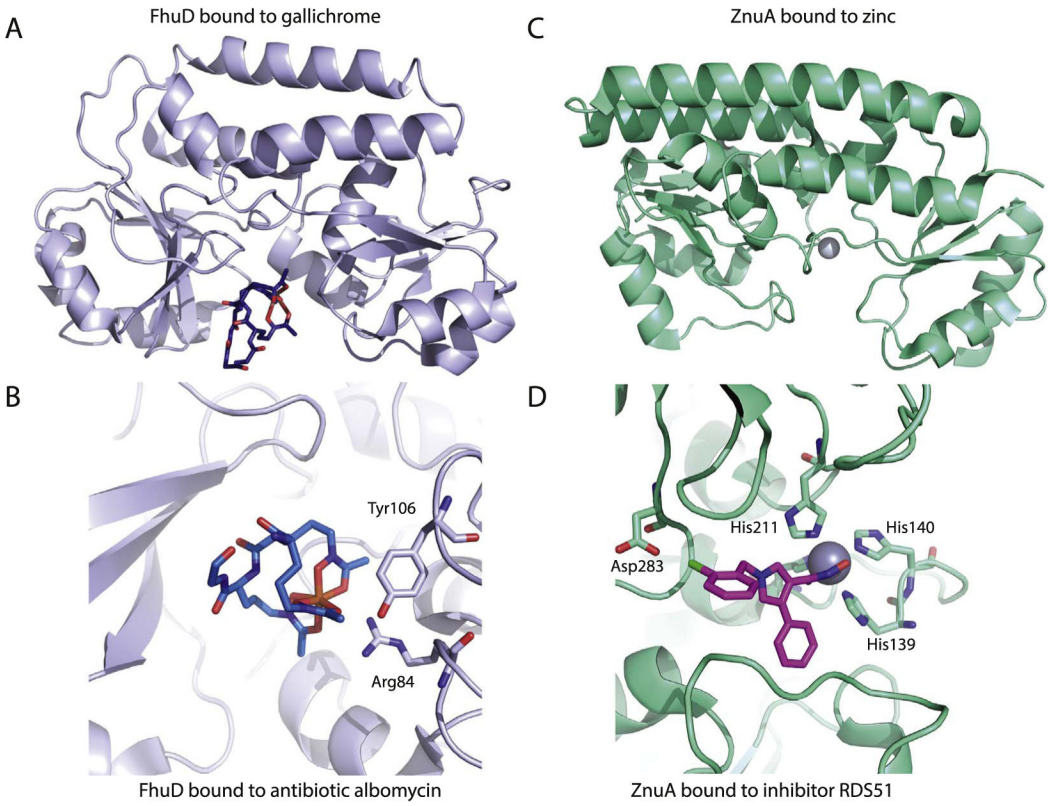


Fig. 2.

Substrate mimics that target ABC importer substrate binding proteins are promising targets for current therapies. (A) Crystal structure of FhuD bound to gallichrome, a ferrichrome analog (PDB ID: 1EFD), and (B) antibiotic albomycin (PDB: 1K7S). Substrates, gallichrome and albomycin, are dark blue and blue, respectively. (C) Crystal structure of ZnuA bound to zinc, shown as grey sphere (PDB ID: 4BBP) and (D) zinc and inhibitor RDS51 complex, shown as grey sphere and magenta sticks, respectively (PDB ID: 4BBP). For panels B and D, residues involved in inhibitor binding are labeled and shown as sticks.

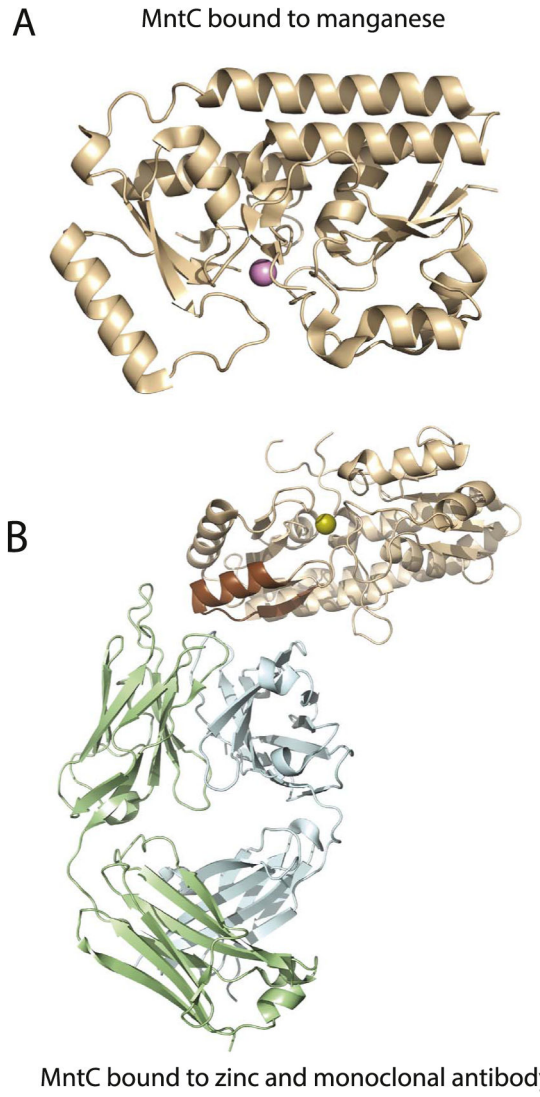
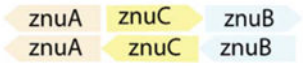
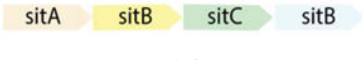
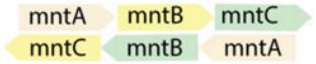
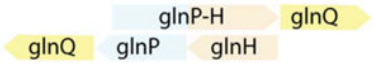
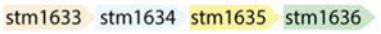
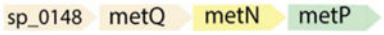


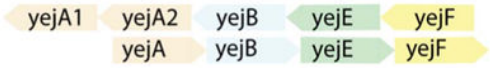
Fig. 3.

Anti-SBP antibodies have proven successful vaccine candidates. (A) Cartoon of crystal structure of MntC bound to manganese, shown as pink sphere (PDB ID: 4K3V) and (B) MntC with Fab fragment for antibody mAB 305-78-7 and iron (PDB ID: 5HDQ). The Fab fragments heavy chain is colored in cyan while the light chain in light green. The residues implicated in antibody binding are colored brown.

Table 1

Select ABC transporters that play a role in full virulence.

Substrate	Name	Organism	Transporter composition*
Metal transporters			
Zinc	ZnuABC	*B. abortus*, *S.* Typhimurium, *C. jejuni*, *M. catarrhalis*, uropathogenic *E. coli*, *A. baumannii*, *Y. pestis*, *P. mirabilis*	
Manganese and iron	SitABCD	Avian pathogenic *E. coli*, APEC O78 strain χ7122, *B. henselae*	 *1, 2*
Manganese and zinc	MntABC	*S.* Typhimurium, *N. gonorrhoea*	
Manganese and zinc	PsaABC	*S. pneumoniae*	
Nickel and cobalt	CntABCDF (formerly Opp1ABCDEF)	*S. aureus*	
Amino acid transporter			
Glutamate	GltTM, SBP (NMB1964)	*N. meningitides*	
Glutamine	GlnHPQ	*S.* Typhimurium, *N. gonorrhoeae*, Group B Streptococci, *S. pneumoniae* (*spd1098–1099*, *spd0411–0412*)	
Alanine	DalS, SBP of putative D-alanine transporter	*S.* Typhimurium	
Cysteine	CtaP, SBP of putative oligopeptide transporter	*L. monocytogenes*	
Lysine, Ornithine	SBP1, SBP3, SBPs putative amino acid transporter	*M. catarrhalis*	*3*
Methionine	MetNIQ	*M. catarrhalis*	
Methionine	MetQNP	*S. pneumoniae*	
Peptide transporter			
Peptides	OppABCDF	*M. catarrhalis* and *B. thruingiensis*	
AMPs	SapABCDF	Nontypable *H. influenzae* and *H. ducreyi*	

Substrate	Name	Organism	Transporter composition*
AMPs	YejABEF	*B. melitensis* (*BMNI_I0006-BMNI_I00010*) and *S.* Typhimurium	

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* Representation from BioCyc Database unless noted [4]. Not all gene directions in operon are represented.