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Review

Diversity of membrane transport proteins for vitamins in bacteria and archaea☆,☆☆

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ABSTRACT

Background: All organisms use cofactors to extend the catalytic capacities of proteins. Many bacteria and archaea can synthesize cofactors from primary metabolites, but there are also prokaryotes that do not have the complete biosynthetic pathways for all essential cofactors. These organisms are dependent on the uptake of cofactors, or at least their precursors that cannot be synthesized, from the environment. Even in those organisms that contain complete biosynthetic pathways membrane transporters are usually present, because the synthesis of cofactors is more costly than uptake.
Scope of review: Here we give an overview of bacterial and archaeal transport systems for B-type vitamins, which are either cofactors or precursors thereof.
Major conclusions: Prokaryotic vitamin transporters are extremely diverse, and found in many families of transporters. A few of these transport systems have been characterized in detail, but for most of them mechanistic insight is lacking.
General significance: The lack of structural and functional understanding of bacterial vitamin transporters is unfortunate because they may be targets for new antibiotics. This article is part of a Special Issue entitled Structural biochemistry and biophysics of membrane proteins. Guest Editor: Bjorn Pedersen.

1. Introduction

Cofactors greatly extend the catalytic potential of enzymes, and allow complex reactions to take place in living cells [1]. B-type vitamins or derivatives thereof constitute a large group of cofactors. Table 1 lists the eight diverse molecules (or groups of molecules) known as B-type vitamins, and the cofactors that are derived from them. The B-type vitamins are essential nutrients for humans, but can be synthesized by many prokaryotes [1]. However, numerous bacteria lack the complete biosynthetic pathways for one or more of these compounds, and therefore depend on their uptake from the environment by membrane-embedded transport proteins. In addition, even the genomes of organisms that encode complete biosynthetic pathways for the vitamins usually also have genes coding for transporters. These organisms can produce vitamins themselves if needed, but probably prefer to take up the compounds when available in the environment, because synthesis requires usually more metabolic energy than transport. For example, 25 mol of ATP are needed for the synthesis of 1 mol of riboflavin [2,3], whereas transport usually costs two ATP or less, depending on the transport system.

Vitamin transporters are essential proteins in many bacteria with incomplete metabolic pathways [4–6], and specific inhibition of the function of these proteins could be a strategy for new antibiotic development. In the past decade the molecular identities of many bacterial vitamin transporters have been revealed. Computational methodologies (comparative genomics, metabolic reconstruction [7]), in combination with classical microbiological and biochemical experiments (e.g. [5]) have played a major role in the recent discoveries, which we will review here.

2. Overview of bacterial solute transport systems

Based on differences in the way solute transport is energized, membrane transporters are classified in three major groups [8]: Primary active transporters, Secondary transporters and Group translocators. In this section we will provide a brief overview of the main characteristics of these three groups.

Abbreviations: ABC, ATP-binding cassette; ECF, energy coupling factor; HET, hydroxyethylthiazole; HMP, hydroxymethylpyrimidine; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; MFS, major facilitator superfamily; Na, nicotinate; Nm, nicotinamide; NR, nicotinamide riboside; NaMN, nicotinate mononucleotide; NMN, nicotinamide mononucleotide; NAD, nicotinamide adenine dinucleotide; SSS, sodium–solute symporters; TMP, thiamin monophosphate; TPP, thiamin pyrophosphate

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Table 1
Overview of B-type vitamins and related cofactors.

Vitamin	Name	Associated cofactor
B_1	Thiamin	Thiaminpyrophosphate
B_2	Riboflavin	FMN/FAD
B_3	Nicotinic acid, Nicotinamide (Niacin); Nicotinamide riboside	NAD^+/$NADP^+$
B_5	Pantothenate	Coenzyme A, phosphopantetheine
B_6	Pyridoxine	Pyridoxal-phosphate
B_7	Biotin	
B_9	Folate	Tetrahydrofolate
B_{12}	Cobalamin	Adenosylcobalamin, methylcobalamin

Primary active transporters comprise many very diverse protein families that use chemical, electrical or solar energy sources to transport substrates across the membrane. Vitamin transporters are found in the largest and most widespread family of primary active transporters: the ATP binding cassette (ABC) transporter family [9,10]. ABC transporters couple ATP hydrolysis to substrate transport. All ABC transporters share the same architecture: Two soluble nucleotide binding or ATPase domains or subunits (NBDs) are located on the cytoplasmic side of the membrane, and two transmembrane domains (TMDs) or subunits are embedded in the lipid bilayer and constitute the pathway for substrate translocation [9,10]. The ATPase domains are conserved in structure and sequence, but the transmembrane domains can adopt different, unrelated structures. Based on the structural diversity ABC transporters have been classified in four different types [11]. These types also differ in details of the transport mechanism. Three of the four types are found exclusively in prokaryotes and are involved in the uptake of nutrients: Type I and Type II importers and ECF transporters. Type I and Type II ABC transporters are dependent on periplasmic or extracellular substrate-binding proteins or domains (SBDs) to bind the transported substrate and deliver it to the transmembrane domains. The substrate is then transported along a pathway at the interface between the two TMDs. Despite these global similarities, the mechanism of transport appears to be very different between the two types [10]. ECF transporters do not make use of soluble SBDs, but instead use one of their TMDs (the S-component) for substrate binding [5,6,12]. The other TMD (The T-component, or EcfT subunit) together with the two NBDs form the so-called ECF module. In many cases the ECF module can associate with different S-components (specific for different substrates, often vitamins) to form a variety of four-subunit complexes, each transporting a different substrate. The fourth ABC transporter type is the exporter, which is found both in pro- and eukaryotes [11,13]. The prokaryotic exporters consist of two NBDs and two TMDs and transport substrate out of cells (or from the inner leaflet of the bilayer to the outer leaflet), and therefore are not likely candidates for vitamin uptake. Nonetheless, a recent study suggests that export-type ABC transporters may in some cases be involved in import functions (see below).

Secondary transporters belong to many different families, with different tertiary structures, oligomeric states and transport mechanisms [13–15]. In bacteria secondary transporters often accumulate substrates in – or deplete them from – cells by coupling substrate transport to the co- or counter-transport of a secondary substrate, frequently Na^+ or H^+. Primary active transporters (such as P-type ATPases) maintain the membrane gradients of the secondary substrate. Some secondary transporters do not catalyze coupled transport, but only facilitate the equilibration of the pools of the substrate on either side of the membrane in a process that is named facilitated diffusion. Secondary transporters usually do not depend on soluble domains or subunits (in contrast to ABC transporters).

Group translocators chemically modify the substrate during the transport reaction [7,16]. The phosphotransferase system (PTS) is the prototypical example of a group translocator, and is used by many prokaryotes for the import of carbohydrates. For each sugar there is a specific membrane-embedded protein consisting of the integral membrane domain (enzyme IIC), which contains the translocation pathway, and two soluble domains (enzymes IIA and IIB), which transfer a phosphate group to the carbohydrate once it has reached the cytoplasmic side of the membrane. Sugar transport and phosphorylation by the PTS are strictly coupled. Phosphoenol-pyruvate (PEP) is the ultimate donor of the phosphate group, which is transferred to enzyme IIA via two proteins that are shared by phosphotransferase systems specific for different substrates: HPr and enzyme I. Apart from the sugar PTS several other transport systems have been loosely classified as group translocators. In these systems the phosphorylation of the substrate may not be very tightly coupled to transport, and therefore these systems could also be classified as secondary transporters that catalyze facilitated diffusion. Some bacterial vitamin transporters use such a mechanism of transport (see below). Cytosolic enzymes may then modify the transported substrate, without the need for a strict coupling between transport and modification.

3. Bacterial and archaeal vitamin transporters

In this section we will give an overview (Table 2) of the known or predicted prokaryotic transporters for the eight B-type vitamins listed in Table 1. The diversity of these transport systems is also schematically summarized in Fig. 2.

3.1. Vitamin B_1: Thiamin

Thiamin pyrophosphate (TPP) is the cofactor derived from thiamin. TPP containing enzymes are involved in cleavage of bonds adjacent to carbonyl groups, and rearrangements in which an acetaldehyde group is transferred from one carbon to another [17]. Thiamin consists of a hydroxyethylthiazole (HET) and a hydroxymethylpyrimidine (HMP) moiety (Fig. 1). The synthesis of thiamin from these compounds is conserved in archaea, bacteria and eukaryotes, whereas the biosynthetic pathways for the two precursors differ substantially [17,18]. Genes for the biosynthesis and transport of thiamin and its precursors have been identified by the presence of the thiamin regulatory RNA element (*THI* element), which operates as TPP-responsive riboswitch [13,18,19]. Missing parts of the biosynthesis pathways allowed for the prediction of substrates for the putative transporters [13,20].

3.1.1. Experimentally characterized thiamin transporters

3.1.1.1. ThiBPQ. ThiBPQ is an ABC transporter for thiamin in *Escherichia coli*. It consists of the substrate-binding protein ThiB, the transmembrane domain ThiP and the NBD ThiQ. Thiamin uptake activity of *E. coli* [20–22] was assigned to this transporter, which is encoded by the *sfuABC* genes in *E. coli* and the *thiBPQ* genes in *Salmonella typhimurium* [18,23]. The structure of the entire ThiBPQ complex is not known, but it is likely to be a Type I ABC importer. The substrate specificity was determined by structural and functional analysis of ThiB, which has a characteristic fold for substrate-binding proteins from ABC transporters, and belongs to cluster D according to the structural classification of Berntsson et al. [20,24–26]. The structure of the protein was solved with thiamin monophosphate (TMP) bound (Fig. 3). ThiB binds TMP, thiamin and thiaminpyrophosphat (TPP) with very similar dissociation constants in the range of 2.3–7.4 nM [13,25]. It must be noted that Hollenbach et al. found a much weaker affinity of ThiB for thiamin (K_D of 0.8 μM) [24,27], which is difficult to reconcile with most of the other available data. Analysis of thiamin transport into *E. coli* revealed a K_M of 15.2 nM. The observed first order rate constant of $1.9 \times 10^{-4}\ s^{-1}$ shows that ThiBPQ transports only 1 molecule of a thiamin per 90 min [28,29].

Table 2
Overview of bacterial and archaeal transport systems for B-type vitamins.

Substrate	Name	Transporter type	Transporter family	Other names	References
Thiamin	ECF-ThiT	ABC transporter	ECF transporter	YuaJ, lmo1429	[5,13,30,31,122]
	ThiBPQ	ABC transporter	Type I ABC importer	*tbpA* for the SBP *sfuABC* (*E. coli*)	[23,24]
	PnuT	Putative facilitator	Pnu type of transporter		[7,13]
	NiaP	Secondary transporter	MFS		[28]
	ThiV	Secondary transporter	SSS		[13]
	ThiT1/ThiT2	Secondary transporter	MFS		[13]
HMP	CytX	Putative secondary transporter	Unknown (homologous to NCS1 family)		[13]
	YkoEDC	ABC transporter	ECF transporter	(*thiUVWX**)	[13]
	ThiXYZ	ABC transporter	Type I ABC importer		[13,18,20]
Thiazole	ECF-ThiW	ABC transporter	ECF transporter		[5,13]
	ThiU	Secondary transporter	Putative MFS		[13]
Riboflavin	ECF-RibU	ABC transporter	ECF transporter	FmnP, *ypaA* (*B. subtilis*)	[5,41,44]
	RibM	Putative facilitator	Pnu type of transporter	PnuX	[5,44,46]
	RibN	Unknown	Unknown		[49]
	RfuABCD	ABC transporter	Probable Type I ABC importer	TP0298	[48]
	RfnT	Secondary transporter	MFS	AraJ, *mlr8412*	[44]
	RibXY	Putative ABC transporter	Putative Type I ABC importer		[52]
	ImpX	Unknown	Unknown		[44]
Niacin and Nicotinamide riboside	ECF-NiaX	ABC transporter	ECF transporter		[5,59,60]
	PnuC	Putative facilitator	Pnu type of transporter		[60]
	NiaP	Secondary transporter	MFS		[28]
	NiaY	Unknown	Unknown		[60]
Pantothenate	ECF-PanT	ABC transporter	ECF transporter		[5]
	PanF	Secondary transporter	SSS		[78,85]
Pyridoxine	ECF-PdxU	ABC transporter	ECF transporter		[5]
	ECF-PdxU2	ABC transporter	ECF transporter		[5,35]
Biotin	ECF-BioY	ABC transporter	ECF transporter	BioMNY	[98]
	YigM	Secondary transporter			[99,101]
Folate	ECF-FolT	ABC transporter	ECF transporter		[106]
	FBT	Secondary transporter	MFS		[109]
Cobalamin	BtuCDF	ABC transporter	Type II importer		[115,116]
	ECF-CbrT	ABC transporter	ECF transporter	CbrTUV	[5]
	Rv1819c	ABC transporter	Exporter		[118]
	BtuM	Unknown	Unknown		[119]
	BtuN	Unknown	Unknown		[119]

* The name *thiUVWX* was proposed instead of *ykoEDC* by Shyns et al. [32] and used later in a few publications (e.g. [122]). The use of the name *thiUVWX* is confusing since different thiamin transport systems are already referred as ThiU, ThiV, ThiW and ThiX. We recommend the use of Yko(F)EDC for this ECF transporter.

3.1.1.2. ThiXYZ. ThiXYZ is another ABC transporter related to thiamin uptake consisting of ThiY (the SBD), ThiX (the TMD) and ThiZ (the NBD) [1,13,28]. The genes *thiXYZ* are found in organisms of several different taxonomic divisions and are always preceded by a *THI* regulatory element, except in *Thermotoga maritima*. Three observations indicate that HMP rather than thiamin is the substrate of the transporter. First, the transporter sometimes occurs in organisms lacking the HMP biosynthetic pathway such as *Brucella melitensis* [1,5,13]. Second, the binding protein ThiY is similar to enzymes for the HMP biosynthesis in yeast. Third, the *thiXYZ* genes co-localize with a HMP kinase gene in the genomes of many organisms [2,3,7,30,31].

ThiY binds formylaminopyrimidine (FAMP) a precursor of HMP with a K_D of 200 nM [4–6,13,18] (Fig. 3), suggesting that ThiXYZ indeed is a FAMP transporter. FAMP is deformylated in the cytoplasm by the aminohydrolase YlmB and then hydrolyzed by TenA forming HMP [7,13,18]. The structure of ThiY from *Bacillus halodurans* was solved at 2.4 Å resolution [5,20]. The protein contains a bound FAMP molecule in a position similar to TMP bound to ThiB [8,20,32]. The pyrimidine moiety of FAMP overlaps with the thiazole ring of TMP and the formyl group with the phosphate group of TMP. A sequence alignment of several ThiY homologues revealed that only residues interacting with the pyrimidine ring are conserved, whereas interaction partners for the formylamino group vary.

ThiXYZ is involved in a thiamin salvage pathway from degraded forms of thiamin present in soil [9,10,13,18]. It has been speculated that several closely related ThiXYZ transporters exist for the uptake of different degradation products containing an intact pyrimidine ring [9,10,13,20,33]. Alternatively, a single transporter may accept different substituted pyrimidines present in the soil as substrates for the synthesis of HMP.

In some organisms the genes for the ATPase subunits (NBDs) of ThiBPQ and ThiXYZ are not encoded in the same operon as the TMDs and the substrate-binding protein [11,13,34]. In organisms of the *Thermus*/*Deinococcus* group *thiQ* is absent and *B. melitensis* and *Agrobacterium tumefaciens* lack the *thiZ* gene. In these cases the ATPase subunit of a different ABC transporter may complement the incomplete ABC transporters. Such exchangeability has been observed for other ABC transporters [10,13,27].

3.1.1.3. NiaP. The gene *niaP* codes for a secondary transporter of the major facilitator superfamily (MFS), of which members are widely spread among pro- and eukaryotes [5,6,12,17,28]. In *Thermus thermophilus niaP* is located in a *THI* regulated operon that also contains the thiamin biosynthesis genes *thiC* and *thiD*. Analysis of the substrate specificity revealed that NiaP has a role as a thiamin uptake system in *T. thermophilus* and does not accept HMP or thiazole as substrates [11,28]. A more detailed description of the NiaP family will be given in the section on Niacin uptake below, because most members of the NiaP family are involved in transport of nicotinate rather than thiamin.

3.1.1.4. ECF-ThiT. Many Firmicutes contain an S-component for thiamin uptake (ThiT). ThiT associates with an ECF module (EcfAA′T, in which EcfA and EcfA′ are the NBDs and EcfT is the second TMD), thus forming a complete ECF-type ABC transporter [5,14,15]. The *thiT* gene is usually preceded by a *THI* regulatory element. The structure of ThiT from *Lactococcus lactis* has been determined and the binding of thiamin and analogues has been studied. ThiT binds thiamin, TMP and TPP with high affinity (low nanomolar or subnanomolar K_D values) [16,30,31].

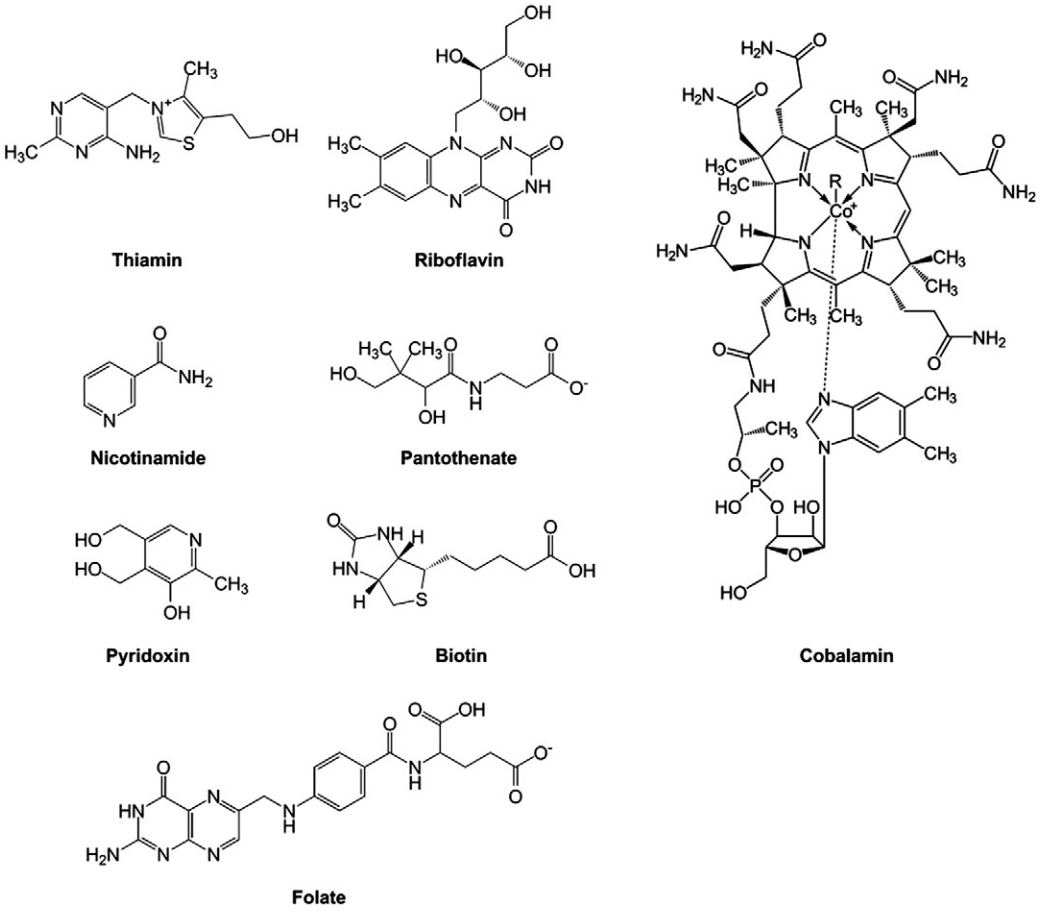

Fig. 1. Chemical structures of B-type vitamins.

3.1.1.5. YkoEDC (ECF-ykoE). A different ECF transporter involved in transport of thiamin or its precursor HMP, termed YkoEDC, is found in some Gram-positive organisms and archaea [5,13,17]. The two NBDs are encoded in tandem in a single gene (*ykoD*), the transmembrane T-component (EcfT) is encoded by *ykoC* and the substrate-binding S-component by *ykoE*. In *Bacillus subtilis* there is an additional gene *ykoF* present in the operon (*ykoFEDC*), which will be discussed separately below. The *ykoEDC* gene cluster is preceded by a *THI* regulatory element and co-localizes with genes involved in thiamin biosynthesis [13,19]. In genomes lacking both the HMP and HET biosynthetic pathways *ykoEDC* is always present together with a putative transporter for HET suggesting that YkoEDC has a role in HMP uptake [13]. Mutational analysis experimentally confirmed YkoFEDC from *B. subtilis* to be a transporter for thiamin or its precursor HMP [21,22,32]. In the same study the presence of HMP only influenced the expression levels of *ykoFEDC* but not that of the other thiamin transporter ECF-ThiT, which supports the role of Yko(F)EDC as a HMP transporter. The positional clustering of *ykoEDC* with *tenA* a gene encoding a thiaminase is an additional indication of this role [13,23].

3.1.1.6. YkoF. YkoF is a soluble protein that binds HMP/thiamin. The gene is present in some operons for ABC and ECF transporters, which are involved in uptake of thiamin precursors. In *B. subtilis* it is localized in the *ykoFEDC* operon and in *Mesorhizobium loti* as well as in *T. maritima* in the *ykoF-thiXYZ* operon [13,24–26,33]. YkoF is not related to substrate-binding proteins (SBDs) associated with Type I and Type II ABC transporters or S-components from ECF transporters (such as the SBD ThiY or the S-component YkoE). Crystal structures of YkoF from *B. subtilis* and *T. maritima* have been determined. YkoF from *T. maritima* consists of four ferredoxin-like protomers and YkoF from *B. subtilis* is a homodimer of protomers with two tandem ferredoxin-like domains. The protein from *B. subtilis* has two substrate binding sites per protomer with different affinities for thiamin (K_D values of 10 μM and 250 μM) [25,34]. Each protomer of YkoF from *T. maritima* has a binding site for thiamin (K_D value of 1.6 μM) [33]. In both structures of YkoF the HMP moiety of thiamin was well resolved. In the YkoF structure of *T. maritima* residual electron density suggested that a mixture of different pyrimidine derivatives was bound in the crystals. Because *ykoF* is found in the *ykoF-thiXYZ* operon it can be speculated that YkoF serves as a cytoplasmic acceptor for a variety of pyrimidine derivatives taken up by the ThiXYZ and YkoEDC transporters.

3.1.2. Predicted thiamin-related transporters

3.1.2.1. ECF-ThiW. ThiW is predicted to be the S-component of an ECF transporter for the thiazole precursor of thiamin [13,24]. The transporter is found in different taxonomic groups (*Firmicutes*, *Archaea* or *Chloroflexi*). The thiamin-related function of the protein was predicted based on the presence of a *THI* element upstream of the gene, and the substrate specificity for thiazole is predicted by co-localization of *thiW* with *thiM*, a gene that codes for a hydroxyethylthiazole kinase [17,29]. Additionally, in *Thermoanaerobacter tengcongensis* and *Streptococcus pneumoniae* a biosynthetic pathway for HET is missing, which might be compensated by the function of ThiW as a thiazole transporter [13].

3.1.2.2. Ecf-HmpT. HmpT was originally predicted to be an S-component specific for hydroxymethylpyrimidine, but was later reclassified as putative pyridoxin-specific S-component [6,35].

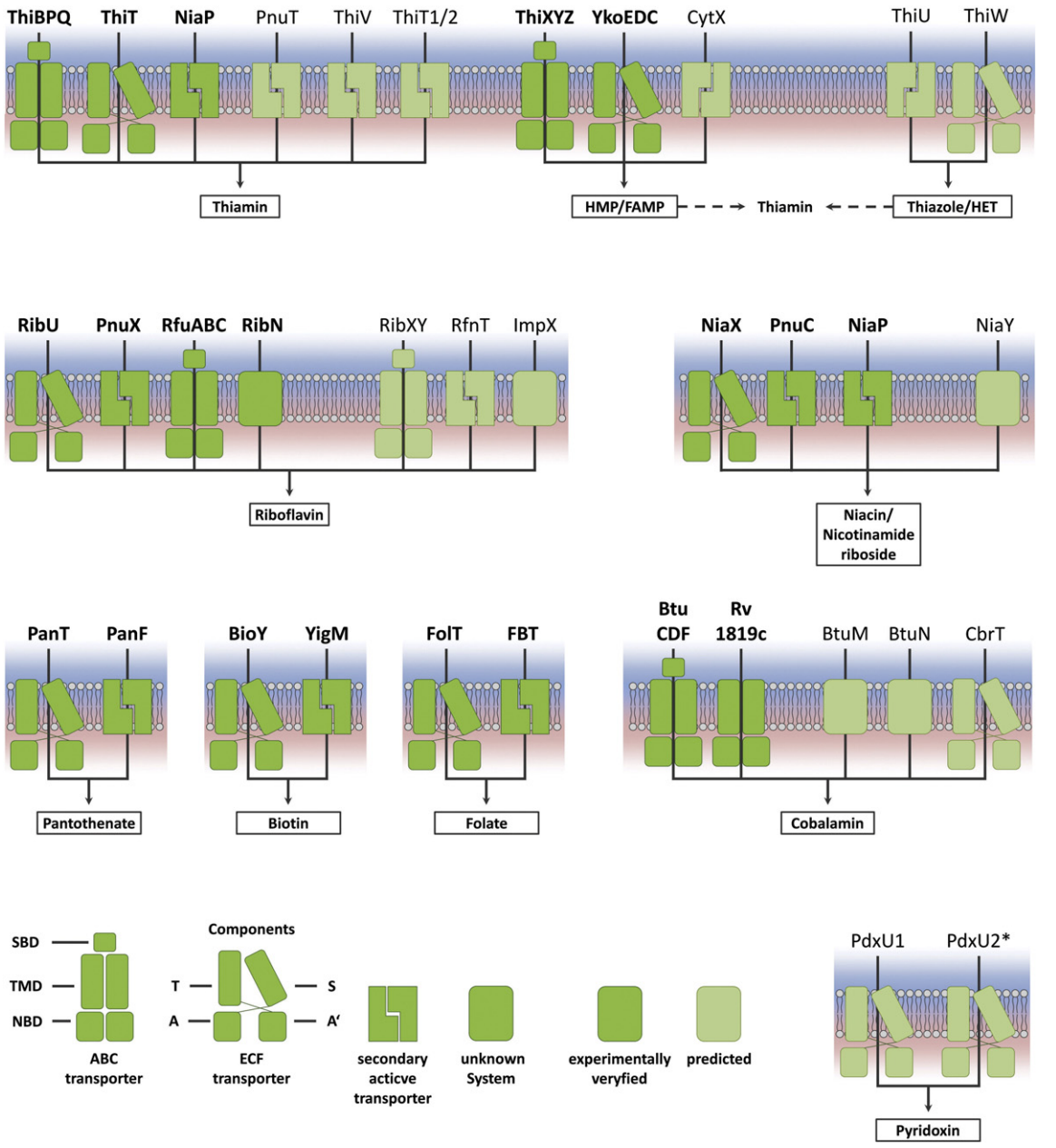

Fig. 2. Overview of bacterial and archaeal transport systems for B-type vitamins. The type of transporter is indicated by the shape and the status of verification by the color. *was initially identified as HmpT but later renamed to PdxU2 [35].

3.1.2.3. Secondary transporters: CytX, ThiU, ThiT1/ThiT2, and ThiV. A putative secondary transporter for thiamin-related compounds CytX was identified in e.g. *Neisseria meningitidis*, *Mannheimia haemolytica*, some pseudomonads and pyrococci [13]. The *cytX* gene always clusters with genes of the thiamin biosynthesis machinery and/or is preceded by a *THI* element. Like for *thiXYZ*, organisms that harbour *cytX* sometimes lack the pathway for HMP synthesis suggesting a function of CytX as a HMP transporter [7]. CytX has 12 putative transmembrane helices and belongs to the Nucleobase Cation Symporter 1 (NCS1) family of secondary transporters. The similarity to transporters for pyrimidine supports the predicted substrate specificity for HMP-related metabolites [7,13].

There are several candidate genes for secondary transporters for thiamin-related compounds that show homology to transporters of the major facilitator superfamily (MFS). ThiU is found in some Pasteurellaceae like *Haemophilus influenzae*. These organisms are not able to synthesize HMP or HET and *thiU* always clusters with the *thiMDE* operon coding for enzymes involved in the biosynthesis of TMP from HMP and HET. The presence of ThiXYZ in these organisms, a transporter for HMP-related molecules, suggests that ThiU is a transporter for HET-related compounds [13].

In three *Thermoplasma* genomes the first archaeal *THI* element regulated genes were identified. They encode two putative transmembrane proteins termed ThiT1 and ThiT2 [13] and show homology to MFS transporters. Based on the presence of the *THI* element they are predicted to be thiamin transporters [13].

Based on localization directly downstream of a *THI* element a gene encoding a membrane protein with 13 putative membrane-spanning segments was predicted to be a thiamin-related transporter in *Methylobacillus flagellatus* and named ThiV [13]. The protein is homologous with the sodium pantothenate symporter PanF (see below), and has been classified as a member of the SSS (sodium solute symporter) family of secondary transporters. These transporters are structurally classified as LeuT like transporters [36].

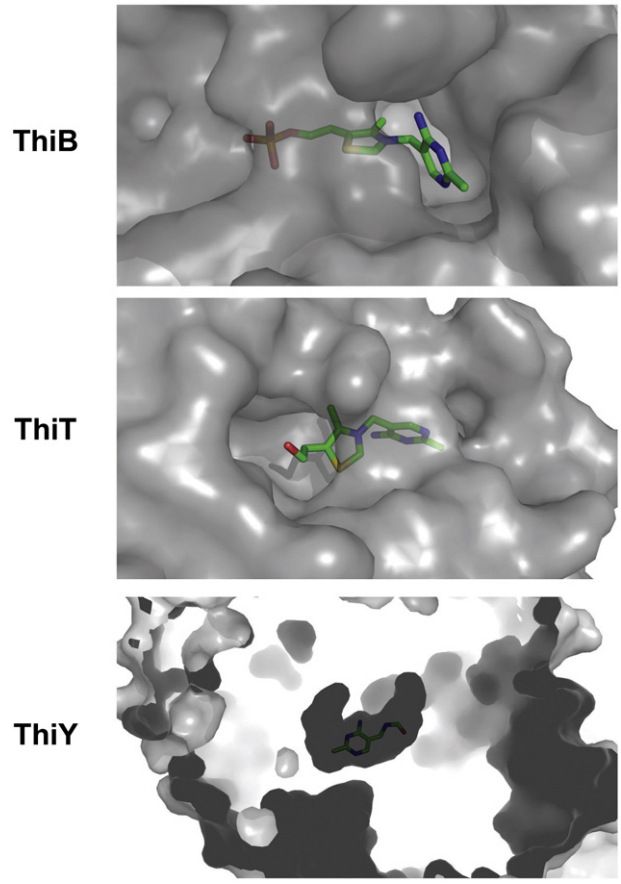

Fig. 3. Substrate binding to ThiB (PDB ID 2QRY), ThiT (PDB ID 3RLB), ThiY (PDB ID 3IX1); The orientation of the substrate molecules bound to thiamin-related transporters. ThiB binds thiaminmonophosphate with the pyrimidine ring facing the pocket opening, whereas the S-component ThiT (ECF transporter) binds thiamin with the hydroxyethyl moiety facing the opening. The structure of ThiY shows the bound FAMP molecule in an occluded state. The protein is displayed as transparent gray surface. The bound ligands are in green (C-atoms), blue (nitrogen), red (oxygen), orange (phosphate) and yellow (sulfur).

3.1.2.4. Pnu type transporter: PnuT. Many Proteobacteria and organisms from the Bacteroidetes/Chlorobi group contain a gene under the control of a *THI* regulatory element, which codes for a protein related to the nicotinamide riboside transporter PnuC and the riboflavin transporter PnuX [7], which will be discussed below. The gene called *pnuT* is located in a conserved cluster together with genes for a TonB dependent outer membrane receptor and a kinase. In *Glaciecola nitratireducens* the kinase is homologous to the thiamin kinase YcfN from *E. coli*. Because Pnu type transporters transport unphosphorylated molecules, which are subsequently trapped in the cytoplasm by phosphorylation (see below), PnuT is predicted to transport thiamin across the membrane, which is then transformed to TMP by the corresponding kinase. An exception is found in *Helicobacter pylori*, where *pnuT* clusters only with the thiaminase gene *tenA*, which is usually involved in the salvage of thiamine precursors [17]. Because the thiazole kinase ThiM, the HMP kinase ThiD and the TMP synthase ThiE, which are responsible for thiamin synthesis from HET and HMP, are present in *H. pylori*, but HMP or HET transporters are absent, it is possible that PnuT has specificity for one of the thiamin precursors in this organism [13].

3.2. Vitamin B_2: Riboflavin

Riboflavin [7,8-dimethyl-10-(1′-D-ribityl)isoalloxazine] or Vitamin B_2 is the precursor of the flavin coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). They are formed in consecutive reactions catalyzed by the riboflavin kinase, which phosphorylates riboflavin forming FMN and the FAD synthase producing FAD from FMN. These enzyme activities can be present in a single peptide chain or in separated proteins. The binding of the nucleotides to proteins produces flavoproteins, which are very abundant (1 to 3% of the proteins encoded in genomes of prokaryotic and eukaryotic origin [37]). The ability of flavins to carry one or two electrons makes them a versatile cofactor for redox reactions [38]. Moreover, flavins are necessary for specialized processes like phototropism [39] or iron acquisition [40].

Their importance for central metabolic processes makes them essential for all living organisms. The majority of prokaryotes can synthesize riboflavin, but also possesses transport systems for uptake.

3.2.1. Experimentally characterized riboflavin transporters

3.2.1.1. ECF-RibU. Many Firmicutes and Thermotogae as well as some Actinobacteria and Archaea contain an S-component for riboflavin uptake (RibU), which assembles into a complete ECF transporter by association with an ECF module (EcfAA′T). The *ribU* gene is usually preceded by a FMN riboswitch regulatory element (also named RFN element) [5]. The structure of RibU from *Staphylococcus aureus* has been determined and the binding of riboflavin to RibU from *L. lactis* has been studied [41,42]. RibU binds riboflavin, FMN and the antibiotic roseoflavin with high affinity (low nanomolar K_D values) [41]. RibU was originally classified as a secondary transporter, but was later found to be the S-component of an ECF transporter [5,43].

3.2.1.2. PnuX or RibM. PnuX/RibM is a transporter for riboflavin found exclusively in Actinobacteria. The name PnuX emphasizes that the protein is related to the family of Pnu type transporters (like PnuT mentioned above, and discussed in more details below), whereas RibM refers to the function of the protein as a riboflavin transporter. PnuX proteins are predicted to have 5–7 membrane spanning helices, and are not related to RibU. PnuX was identified as a putative riboflavin transporter by a comparative genomics approach, since the gene is often located in the riboflavin biosynthesis operon *ribBAHM*, which is preceded by an FMN regulated riboswitch [44]. It was shown experimentally that RibM is a riboflavin transporter by the uptake of ^{14}C-labeled riboflavin into *E. coli* harbouring a plasmid encoding *pnuX* from *Corynebacterium glutamicum* or *Streptomyces davawensis* [45,46]. A K_m value of 11 μM for riboflavin was reported. Uptake assays revealed that roseoflavin is also a substrate, but FAD is not. It is not clear whether FMN is a substrate because the two reports show contradictory results. A facilitated diffusion mechanism was proposed as transport mechanism [46]. Directionality would be then achieved by intracellular metabolic trapping, which would be a phosphorylation by a flavokinase [47].

3.2.1.3. RfuABCD. An SBD-dependent ABC transporter for riboflavin (encoded by the *rfuABCD* operon) was identified in different spirochetes [48]. The periplasmic lipoprotein RfuA (the SBD) from the riboflavin auxotroph *Treponema pallidum* binds riboflavin. A crystal structure of RfuA at 1.3 Å resolution revealed a different binding mode for the substrate compared to RibU (Fig. 4). Apparently different binding strategies for identical substrates have evolved. The different orientation of the bound molecule is likely to contribute to different substrate specificities. For RibU the ribityl chain is positioned toward the extracellular opening of the binding site [42]. This orientation tolerates the phosphate group present in FMN, for which a dissociation constant of 36 nM was determined [41]. For RfuA the ribityl part is oriented toward the center of the protein, where it is difficult to accommodate an additional phosphate group (Fig. 4). Based on the analysis of residues involved in riboflavin binding to RfuA 32 homologous binding proteins were predicted to be specific for riboflavin [48]. This example shows how structural data of a single transporter (instead of genomic comparison) can also be used to identify the substrate of a transporter.

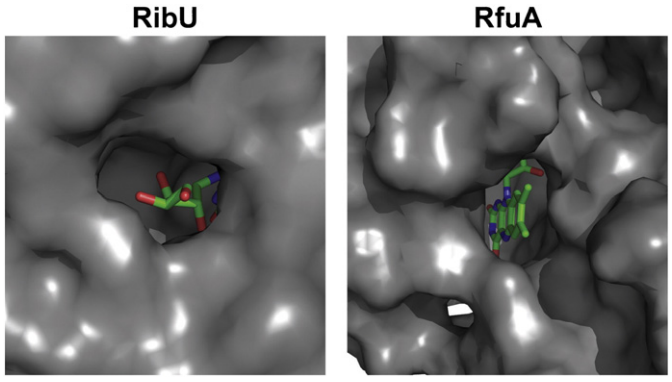

Fig. 4. Riboflavin binding to RibU (PDB ID 3P5N) and RfuA (PDB ID 4IIL). The orientation of riboflavin located in the binding pockets of RibU and RfuA. In the S-component RibU riboflavin is positioned with its ribityl chain toward the opening. In RfuA the methyl substituents of the isoalloxazine ring are exposed through the opening. The protein is displayed as gray surface. The bound ligands are in green (C-atoms), blue (nitrogen), red (oxygen).

3.2.1.4. RibN. The most recently identified riboflavin transporter is RibN from *Rhizobium leguminosarum* (RL1692) [49]. An FMN riboswitch upstream of the RL1692 gene indicated that the encoded membrane protein is involved in riboflavin translocation [44]. Homologues are distributed among α-, β- and γ-proteobacteria, although the corresponding regulatory riboswitch is present only in organisms of the α-subdivision [49]. The study revealed that RibN from *R. leguminosarum*, *Ochrobactrum anthropi* (α-proteobacteria) and *Vibrio cholerae* (γ-proteobacteria) mediates uptake of riboflavin and FMN. However, it is not clear whether FMN is a transported substrate or whether there are periplasmic phosphatases present that hydrolyze FMN to riboflavin, which is then taken up. In silico analysis of RibN homologues revealed 7–9 putative membrane spanning segments. RibN shows no sequence similarity to any characterized transporter, but has Eam-like domains which are found in some secondary transporters [50,51].

3.2.2. Putative riboflavin transporters impX, rfnT, and ribXY

Based on the presence of FMN riboswitches or co-localization with riboflavin biosynthesis genes, three more genes have been predicted to encode riboflavin transporters: *impX*, *rfnT* [44] and *ribXY* [52]. So far, no experimental evidence exists for the predicted role in riboflavin uptake. Homologues of ImpX are found in different bacteria (e.g. Firmicutes, Proteobacteria, Actinobacteria) and archaea and are often annotated to belong to the diffuse drug metabolite family of secondary transporters (DMT family [51]). The FMN riboswitch upstream of the *impX* gene is found only in the organisms *Fusobacterium nucleatum* and *Desulfitobacterium hafniense*. Recently, RibXY from different Chloroflexi species was proposed to be an ABC type riboflavin transporter [52], although the NBDs are not encoded in the same operon as the *ribXY* genes. The *ribXY* genes are preceded by an FMN riboswitch and the RibXY system shows similarity to the ThiXYZ system that transports HMP. Exchangeable NBDs from other ABC transporters may be used to make the transporter complete ([27], see above). No regulatory element was found upstream of *rfnT*, which was predicted to encode a riboflavin transporter based only on genome localization in three different *Rhizobium* genomes (*M. loti*, *Sinorhizobium meliloti*, *A. tumefaciens*). The gene encodes a membrane protein of 11 membrane-spanning segments and is homologous to MFS transporters [44].

3.3. Vitamin B_3: Niacin (nicotinic acid — Na, nicotinamide — Nm), Nicotinamide riboside — NR

The name niacin is used for two distinct precursors of NAD^+: nicotinic acid (Na) and nicotinamide (Nm), however in literature the definition is not consistent and nicotinamide riboside (NR) is sometimes included as well [53]. NAD^+ and the related reduced and phosphorylated compounds (NADH, $NADP^+$ and NADPH) act as coenzymes for enzymatic hydride-transfer reactions [54]. NAD^+ is also a substrate in different enzymatic reactions like NAD^+-dependent protein deacetylases (Sirtuins), poly(ADP-ribose) polymerases or bacterial DNA ligases [53,55]. NAD^+ is also an important signaling molecule of the cellular redox status [53]. The biosynthesis of NAD^+ is very complex, based on different de novo and salvage pathways. Several biosynthetic modules, none of which is universally conserved, are used in a combinatorial way [56]. Although several exceptions exist the de novo biosynthesis routes using aspartate are found in prokaryotes and the pathway starting from tryptophan is used by eukaryotes [56]. Most of the salvage pathways start with niacin, NR or nicotinate riboside (NaR), the deamidated NR molecule [54]. These molecules, containing a pyridine base as the core structure, are the transported forms of Vitamin B_3, thus niacin transporters are important parts of the NAD^+ biosynthesis machinery.

Active transport of niacin by bacteria has been observed for decades [57,58], but transport systems were not identified until recently.

3.3.1. ECF-NiaX

Many Firmicutes contain an S-component for niacin uptake (NiaX), which assembles into a complete ECF transporter by association with an ECF module (EcfAA′T) that is often used by other S-components as well [5,59]. The purified ECF-NiaX transporter from *L. lactis* has been reconstituted into liposomes, and it was shown to support ATP dependent uptake of nicotinic acid [59].

3.3.2. NiaP

NiaP is a member of the major facilitator superfamily (MFS) of secondary transporters. The gene occurs in different regulons for NAD^+ metabolism (controlled by the transcriptional regulators NadR, NiaR and NrtR) but also in the absence of a regulatory element [28,60]. Orthologues are found in all kingdoms of life including humans (SVOP) [61] and plants [60]. This is a notable exception to the observation that eukaryotes tend to use different transport systems for vitamins compared to prokaryotes. The NiaP proteins are predicted to have 10 membrane-spanning helices and display the highest similarity to the benzoate transporter BenK (*Acinetobacter* sp.) and the 4-hydroxybenzoate transporter PcaK (*Pseudomonas putida*) [60,62,63]. A niacin auxotroph *E. coli* showed improved growth in the presence of limiting amounts of Nm when *niaP* from *B. subtilis* was expressed recombinantly [60]. Similar experiments confirmed the role of NiaP in *Acinetobacter baylyi* [64]. Both studies observed a residual transport activity at niacin concentrations above 100 μM, which could be attributed to an unknown low-affinity niacin permease or to free diffusion of Nm across the membrane [4]. In a more extensive study using *L. lactis* as expression host numerous *niaP* homologues were tested in whole cell uptake assays. An energy-dependent bidirectional transport model of niacin transport was proposed for NiaP from *B. subtilis* and nicotinate transport activity was confirmed for homologues from *Burkholderia xenovorans* and *Ralstonia solanacearum*, mouse (SVOP), *Arabidopsis thaliana* and maize [28].

NiaP from *B. subtilis* has a preference for the deamidated forms of niacin (such as nicotinate or nicotinate mononucleotide) [28]. Transport of the Nm, NMN, NAD^+ or phosphorylated derivatives was not observed. Uptake of the deamidated mononucleotide NaMN was observed at high concentrations, but was attributed to a partial hydrolysis and subsequent transport of Na. NiaP has narrow substrate specificity, since its *N*-methyl derivative trigonelline was not transported, with the exception of the NiaP homologue from *A. thaliana* where it appears to be the physiological substrate [28]. A NiaP homologue from *T. thermophilus* has been shown to be specific for thiamin rather than niacin, which is in accordance with the genomic organization of the *niaP* gene in an operon for thiamin biosynthesis in this organism (see section on thiamin above).

3.3.3. PnuC

PnuC belongs to the Pnu-type transporter family and is found in many bacterial taxonomic divisions. Homologues from *H. influenzae*, *E. coli* and *Salmonella enterica* have been functionally characterized [4,65–67]. The proteins have 8 predicted membrane-spanning segments with both termini located in the cytoplasm [67]. The transporter was initially thought to transport NMN. This hypothesis was based on uptake of NMN carrying a radioactive label on the phosphate as well as at the NR moiety [68]. Later studies revealed that PnuC accepts exclusively the unphosphorylated NR [4,69,70] and only a mutant version of PnuC is capable of transporting NMN [70]. The exact transport mechanism has not been unraveled, but transport of NR is not coupled to a concentration gradient of a second solute (as observed in many secondary transporters) or ATP hydrolysis (as observed for ABC transporters). It has been shown that transport is strongly influenced by an intracellular kinase activity that converts NR to NMN [71,72]. This activity results from the cytoplasmic enzyme NadR, which contains a NR kinase activity as well as a NMN adenylyl transferase activity (NMNAT) [73,74]. An inactive kinase domain inhibited uptake of NR almost completely defining the substrate phosphorylation as an essential step in the directed substrate transport [72]. This observation could be indicative of a mechanism similar to group translocators. The NMNAT activity of NadR is not critical for PnuC mediated substrate translocation [66]. Although a group translocation mechanism is possible, there is no evidence for a direct interaction with NadR, and therefore a mechanism based on facilitated diffusion followed by metabolic trapping is also possible [66,72]. For the homologous riboflavin transporter PnuX weak experimental evidence exists for riboflavin export in RF overproducing *B. subtilis* (see PnuX, [46]) and an involvement of PnuC in excretion of pyridines, which is essential under certain growth conditions, has been discussed [46,70]. A vitamin exporting activity would be indicative of a facilitated diffusion mechanism.

3.3.4. NiaY

NiaY is a putative niacin transporter with 9 predicted membrane-spanning segments [60], which shows no sequence similarity to any protein of known function. It has been identified as a niacin transporter because the *niaY* gene is located in the NiaR regulon in *B. halodurans*. *niaY* genes appear in only a few genomes but never in organisms possessing other niacin transporters like NiaP or NiaX. A co-occurrence with *pncB* a gene involved in niacin salvage, supports the proposed function of NiaY as a niacin transporter [60].

3.4. Vitamin B_5: Pantothenate

Pantothenate is a precursor of Coenzyme A, which serves as a cofactor in numerous metabolic reactions related to lipid metabolism, respiration or lignin biosynthesis [75]. The five step biosynthetic pathway of CoA from pantothenate is present in virtually all organisms. However, the de novo biosynthesis of pantothenate from β-alanine and α-ketoisovalerate is only present in some bacteria, fungi and plants [76], reflecting the widespread importance of pantothenate transporters. Transporters for the uptake of pantothenate have been characterized in prokaryotes and eukaryotes [5,77–79].

3.4.1. PanF

A pantothenate uptake activity was discovered in *E. coli* in the early 1970s and attributed to a specific permease termed PanF [78]. The transporter belongs to the SSS family of secondary transporters and is closely related to the proline transporter PutP and the sodium dependent glucose transporter SGLT [80,81]. The transporter consists of 13 membrane-spanning segments with the N-terminus oriented to the periplasm [82]. A structure of the homologous sodium dependent galactose symporter from *Vibrio parahaemolyticus* serves as a structural model for transporters of the SSS family [83,84]. These transporters are structurally classified as LeuT-like transporters [36]. PanF couples the uptake of pantothenate to the symport of sodium with a K_M value for pantothenate of 0.4 μM and for sodium of 0.8 mM [85]. Competition experiments revealed high substrate specificity for pantothenate with pantetheine as the only substance able to inhibit pantothenate uptake. PanF and the mammalian pantothenate transporter SMVT are both members of the SSS family (similarity of 53% and identity of 23%), but the substrate specificity is different, because SMVT accepts also biotin and lipoate [86].

3.4.2. ECF-PanT

Many Gram-positive bacteria and Thermotogae contain an S-component for pantothenate uptake (PanT), which assembles into a complete ECF transporter by association with an ECF module [5]. The *panT* gene often co-localizes with various pantothenate salvage genes. ECF-PanT from *Leuconostoc mesenteroides* has been shown to transport pantothenate when expressed in a β-alanine-auxotrophic, pantothenate transport-deficient *E. coli* strain [87].

3.5. Vitamin B_6: Pyridoxine

Pyridoxine, pyridoxal and pyridoxamine are the compounds that are named Vitamin B_6. These compounds can be converted in pyridoxal-phosphate, which is a cofactor in e.g. transamination reactions. Very little is known about Vitamin B_6 transporters in prokaryotes. The only predicted transporters are ECF-PdxU1 and ECF-PdxU2 [5,6,35]. PdxU1 is an S-component found in Actinobacteria, Archaea, Firmicutes and Thermotogae predicted to bind pyridoxine. PdxU2 (found in Archaea, Firmicutes and Thermotogae) was originally named HmpT, because it was predicted to bind HMP, but was later renamed PdxU2, because genomic context analysis revealed that it was more likely a pyridoxine transporter [35]. There is no experimental data for Vitamin B_6 transport by these proteins.

3.6. Vitamin B_7: Biotin

Biotin is involved in many enzymatic carboxylation, decarboxylation and transcarboxylation reactions and serves as a universal carboxyl group carrier [88]. Biotin-dependent enzymes carry their prosthetic group covalently attached via an amide bond with specific lysine residues [89]. Moreover biotin plays an important role in cell signaling, gene expression and chromatin structure [90]. Its epigenetic role is mediated by the histone biotinylation [91]. Biosynthesis of biotin consists of two parts, the synthesis of a pimelate moiety and the subsequent construction of the bicyclic ring [88]. While the ring assembly is highly conserved in all known biotin-producing organisms, the pimelate precursor is synthesized using different pathways [88,92]. Biotin absorption in mammals is mainly mediated by the multivitamin transporter SMVT (see above) [86], and by Mct1, a member of the monocarboxylate transporter family [93,94]. Fungi possess an H^+-coupled symporter for biotin (Vht1p) [79]. Transporters for biotin in prokaryotes were unknown for a long time, although biotin uptake activity had been observed already in the 1970s [95–97].

3.6.1. ECF-BioY

BioY is the S-component of an ECF transporter [5,98], and is found in many diverse groups of bacteria and Archaea. BioY was implicated in biotin transport based on evidence from gene co-regulation, co-localization, and co-occurrence. Although BioY is an S-component for an ECF transporter in most organisms, the *bioY* gene is also found in some organisms that do not encode an ECF module. In these organisms the solitary BioY protein (without the ECF module) can transport biotin by an unknown mechanism [99]. It must be noted that S-components for other vitamins (such as ThiT and RibU, see above) do not display such a solitary transport function. Moreover, the solitary transport function is not conserved in all BioY proteins [100]. BioY from *L. lactis* and *Rhodobacter capsulatus* have been purified and reconstituted into

liposomes, and did not support transport of biotin as solitary proteins. Surprisingly, BioY from *R. capsulatus* appeared to transport biotin when expressed in *E. coli*, but further experiments are needed to reconcile these different experimental results.

The crystal structure of BioY from *L. lactis*, which associates with a shared ECF module, has been determined. BioY has the same fold as other S-components, and binds biotin with high affinity (low nanomolar K_D) [100].

3.6.2. YigM

Uptake of biotin in *E. coli* (which does not contain an ECF-BioY transporter) has been described for decades, but the activity could not be attributed to a specific protein [95–97]. Analysis of several candidate membrane proteins revealed *yigM* to encode a membrane transporter specific for biotin [101]. This protein is predicted to have 10 membrane-spanning segments with homologues present in different classes of proteobacteria. A Δ*yigM* mutant strains of *E. coli* displays reduced biotin uptake rates, and when *yigM* is overexpressed in *E. coli* highly stimulated biotin uptake activity is observed. A K_M of 74 nM determined for biotin uptake by YigM corresponds well to previously reported values of 50 nM [97], 270 nM [96] and 140 nM [95]. Equally, a V_{max} of 7 pmol/min/mg dry cells determined for YigM mediated uptake is in agreement with previously determined values of 6.6 to 7 pmol/min/mg. Evidence for a specific energy dependence is weak and not consistent between early reports and the recent data for YigM. A minor effect of the uncouplers CCCP and FCCP on the biotin uptake efficiency of YigM (40% reduction by FCCP, 30% by CCCP) is in contrast to a larger effect in earlier reports [95–97]. Because coupling to sodium ions was not observed in uptake assays using *E. coli* membrane vesicles, a proton coupled symport mechanism and a facilitated diffusion mechanism are possible.

3.7. Vitamin B_9: Folate

Folates are molecules that consist of three structurally distinct moieties: pterin, p-aminobenzoate (pABA) and glutamate. A fully oxidized pterin part is not present in naturally occurring folates, which appear exclusively in the dihydro or tetrahydro state [102]. They harbour an additional polyglutamyl tail linked to the γ-carboxyl group of the first glutamate [102]. Tetrahydrofolate is a one carbon acceptor and donor involved in biosynthesis of purins, thymidylate and amino acids like methionine, serine and glycine [103]. Its de novo synthesis is conserved among bacteria, plants, fungi and some protists, and consists of pathways for the pterin (starting from GTP) and the pABA (starting from chorismate) [102,104]. The partly and fully reduced folates can be produced from folate. Mammalian folate uptake systems are diverse and range from different secondary transporters to receptor-mediated endocytosis [105]. Bacteria and plants lack homologues of these transporters but have at least two different folate transporters, one of which is only present in bacteria.

3.7.1. ECF-FolT

FolT is an S-component of ECF transporters found in Gram-positive bacteria and Thermotogae [5]. FolT binds (6S)-folinic acid and folic acid with nanomolar K_D values, but does not bind (6R)-folinic acid (the unnatural isomer) [106]. The structure of the complete FolT-ECF transporter has been solved (in a substrate-free state), and the purified complex, reconstituted in liposomes, supported ATP-dependent uptake of folate [107].

3.7.2. FBT type folate transporters

The FBT family includes transporter homologues from protists, plants and cyanobacteria [108,109]. Members of the FBT family belong to the major facilitator superfamily of secondary transporters which share a common core structure made of 12 membrane-spanning segments with both termini located intracellularly [110,111]. Heterologous expression of the cyanobacterial homologues in *E. coli*, *L. lactis* or yeast yielded insufficient amounts to perform transport assays using radiolabeled substrates in whole cells or membrane vesicles, but growth assays and mutational analysis revealed seven residues that are critical for transport in Slr0642 (FBT of *Synechocystis*) [109]. Two of these residues are conserved among the folate transporters but are missing in the biopterin transporters, indicating that the identified residues contribute to the substrate specificity of members of the FBT family [109]. The related transporters found in proteobacteria have not been characterized.

3.8. Vitamin B_{12}: Cobalamin

Most bacteria and archaea have enzymes that require cobalamin as cofactor, such as the B_{12}-dependent methionine synthase and ribonucleotide reductase, but only some bacterial and archaeal species are able to synthesize the cofactor de novo. Therefore, uptake of cobalamin is crucial for many prokaryotes.

3.8.1. ABC transporters

The best-characterized transporter for cobalamin is the Type II ABC importer BtuCDF from *E. coli*, in which BtuC is the TMD, BtuD the NBD and BtuF the periplasmic substrate-binding protein [112–116]. It is difficult to predict from sequence comparison how widespread related cobalamin transporters are among other prokaryotes [117].

Surprisingly, an ABC transporter of the exporter class appears to be involved in cobalamin uptake in *Mycobacterium tuberculosis* [118]. The transporter encoded by the *Rv1819c* gene is a homodimer, in which each subunit contains tandem transmembrane and nucleotide binding domains. The *Rv1819c* gene is essential for uptake of cobalamin in *M. tuberculosis*. ABC transporters of the exporter class are very abundantly present in prokaryotes, and often have unidentified functions. The import function of Rv1819c could indicate that more ABC exporters may have import functions.

3.8.2. ECF CbrT

CbrT is an S-component of ECF transporters found in Gram-positive bacteria and archaea [5]. It is predicted to be specific for cobalamin, but experimental evidence is lacking.

3.8.3. Predicted transporters: BtuM, BtuN

Comparative genomics analysis of the Vitamin B_{12} utilization in bacteria suggests two further candidate B_{12} transporters. The functional assignment of *btuM* and *btuN* is based on chromosomal clustering with the outer membrane receptor *btuB*, the presence of the B_{12} regulatory element and the prediction of several transmembrane segments [119]. BtuM and BtuN have five and four predicted transmembrane segments, respectively. There is no experimental evidence that these proteins indeed have a role in B_{12} uptake.

4. Conclusion

Despite the crucial importance of vitamin-related molecules in prokaryotes, the knowledge about the uptake of B-type vitamins and their precursors is limited. Comparative genomic approaches have made an enormous contribution in the (tentative) identification of vitamin transporters in bacteria and archaea. Prokaryotes appear to have developed a variety of transport systems for vitamins, which differ in their energy dependence, substrate affinities and taxonomic distribution.

The diversity of bacterial vitamin uptake systems has medical relevance. Cofactor biosynthesis and gene regulation at the level of riboswitches are already targets for the development of new antimicrobial drugs [76,120]. Vitamin transporters could also serve as targets for antimicrobial drugs as they could inhibit growth of pathogenic organisms that are auxotroph for vitamins. For example *Haemophilus influenzae* depends on nicotinamide riboside uptake for survival [4].

As always, potential drugs must selectively target microbes. In many cases the human transport systems are unrelated to the bacterial transporters (except for a few secondary transporters, such as PanF). Therefore, it is likely that there are mechanistic differences between most bacterial and human transport systems for vitamin uptake, which could allow for the development of selective antimicrobial drugs with the transporters as target.

Humans not only take up vitamins that are present in their diet, but also use vitamins produced by the gut microbiome. Food-related lactic acid bacteria and species of the genus *Bifidobacterium* are known vitamin producers in the human colon [104]. Because vitamins are used as exchange currencies between different bacteria in the gut microbiome and between the bacteria and their hosts, bacteria must also contain vitamin efflux systems [104]. However, mechanisms of vitamin export are poorly understood. Knowledge about export could help to improve the industrial vitamin production by prokaryotic strains further [43,121].

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