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Review

Membrane transport metabolons

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ABSTRACT

In this review evidence from a wide variety of biological systems is presented for the genetic, functional, and likely physical association of membrane transporters and the enzymes that metabolize the transported substrates. This evidence supports the hypothesis that the dynamic association of transporters and enzymes creates functional membrane transport metabolons that channel substrates typically obtained from the extracellular compartment directly into their cellular metabolism. The immediate modification of substrates on the inner surface of the membrane prevents back-flux through facilitated transporters, increasing the efficiency of transport. In some cases products of the enzymes are themselves substrates for the transporters that efflux the products in an exchange or antiport mechanism. Regulation of the binding of enzymes to transporters and their mutual activities may play a role in modulating flux through transporters and entry of substrates into metabolic pathways. Examples showing the physical association of transporters and enzymes are provided, but available structural data is sparse. Genetic and functional linkages between membrane transporters and enzymes were revealed by an analysis of *Escherichia coli* operons encoding polycistronic mRNAs and provide a list of predicted interactions ripe for further structural studies. This article supports the view that membrane transport metabolons are important throughout Nature in organisms ranging from bacteria to humans.

Contents

Abbreviations: ABC, ATP-binding cassette; AE1, anion exchanger 1; CAII, carbonic anhydrase II; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; MFS, major facilitator superfamily of transporters; PFK, phosphofructokinase; SLC, solute carrier; STAS, sulfate transporter and anti-sigma factor antagonist; TM, transmembrane

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1. Introduction

The metabolic activity of a cell consists of a set of highly coordinated and highly regulated processes. Metabolic pathways are often organized into metabolons that are weak complexes of sequential enzymes, allowing the efficient channeling of metabolic intermediates from one active site directly to the next. Channeling prevents the release of precious and often labile or toxic intermediates. Channeling also increases metabolic efficiency and provides an opportunity for exquisite control of the flux through a pathway by regulating not only individual enzyme activities but also protein–protein interactions. Recent high-throughput studies [1–4] have identified cellular interactomes; that is, networks and complexes of proteins that carry out highly integrated cellular functions. These interactomes include membrane proteins and their associated proteins. This article provides evidence gleaned from studies of mammalian, yeast, bacterial and other systems that supports the hypothesis that there is a genetic, functional and structural interaction between membrane transport proteins and enzymes, creating membrane transport metabolons that are dynamic complexes of transport proteins and enzymes involved in the metabolism of the transported substrates.

2. Metabolons and substrate channeling

2.1. Metabolons

Paul Srere introduced the concept of the metabolon in 1985 [5–7] based on early work of the organization of glycolytic and Krebs cycle enzymes, particularly of Russian workers such as Kuzin and the team of Kurganov and Lyubarev [8], as well as Clarke working in Australia [9,10]. Metabolons involve the transient association of sequential enzymes to form a complex, typically organized on the cytoskeleton or a membrane [11]. Once released from the cytoskeleton or membrane, the enzyme complexes are fragile and often dissociate into subunits, making it difficult to study the intact complex in isolation.

In cells, the concentration of proteins is orders of magnitude higher than the dilute concentrations typically used in biochemical characterizations in buffer solutions [12]. For example, the concentration of hemoglobin in the red cell is about 350 mg/ml and proteins in bacterial cytosol are of a similar concentration [13]. Similarly, the concentrations of some proteins in biological membranes are very high and they are often clustered in microdomains or lipid rafts [14,15]. Thus, weak binding constants between components of a metabolon may be physiologically relevant, particularly when organized on the 2-dimensional surface of a membrane. Also, the hydration of proteins, which may affect the activity of water, and molecular crowding can influence protein interactions. This leads to the realization that the cytosol of cells, although crowded, is highly organized and not simply a dilute solution where random interactions take place.

2.2. Substrate channeling

Strong evidence for substrate channeling comes from structural studies of enzyme complexes [16,17] such as bacterial tryptophan synthase that consists of an $\alpha_2\beta_2$ tetramer (Fig. 1). Each α subunit active site is connected to a β subunit active site by a 25 Å long "hydrophobic channel" that likely facilitates the diffusion of indole directly from one active site to the next. A second example is the bifunctional enzyme dihydrofolate reductase-thymidylate synthase found in some plant species and protozoal parasites like malaria and trypanosomes (Fig. 1B). This enzyme contains a charged region on the surface of the enzyme that may act as a pathway or "electrostatic highway" that structural [18] and simulation studies [19] have indicated likely to guide a substrate from one active site to another. Other examples of enzymes with characterized substrate tunnels include carbamoyl phosphate synthetase, glutamine phosphoribosylpyrophosphate amidotransferase, asparagine synthetase and oxo-acid dehydrogenases [17].

In plants, the importance of metabolon formation on the cytosolic side of the endoplasmic reticulum membrane and metabolic channeling in the synthesis of natural products such as isoprenoids, alkaloids, flavonoids, and cyanogenic glucosides in secondary metabolism has been highlighted [20]. These metabolons involve soluble proteins. Now we will see that membranes play a key role in the organization of metabolons through the association of specific transport proteins with cytosolic enzymes.

3. A bicarbonate transport metabolon

In 1998, Vince and Reithmeier reported [21] that carbonic anhydrase II (CAII) binds to the carboxyl-terminal tail of human Band 3 (anion exchanger 1, AE1), the erythrocyte Cl^-/HCO_3^- exchanger. The binding site on Band 3 was characterized as a motif that contained a hydrophobic residue followed by an acidic sequence (−LDADD−) [22], which provided a binding site for the N-terminal basic region of CAII but not CAI [23]. The interaction of CAII with Band 3 facilitated bicarbonate transport across the membrane, creating a bicarbonate transport metabolon [24–27] as illustrated in Fig. 2. Indeed, the coupled activity of AE1 and CAII creates a pH microenvironment in the vicinity of the transporter [28]. A functional association of carbonic anhydrase isoforms with other bicarbonate transporters including sodium bicarbonate co-transporters (NBC) has subsequently been reported [29–36]. Other reports [37] acknowledge that CAII plays an important role in CO_2 transport by red blood cells but dispute the nature and significance of the interaction with AE1 in accelerating bicarbonate transport. Rather, CAII is predicted to have a major effect on CO_2 movement across the membrane, not anion exchange itself and in the accelerating local pH changes. A role of carbonic anhydrase in the dissipation of local pH changes by facilitating cytoplasmic H^+

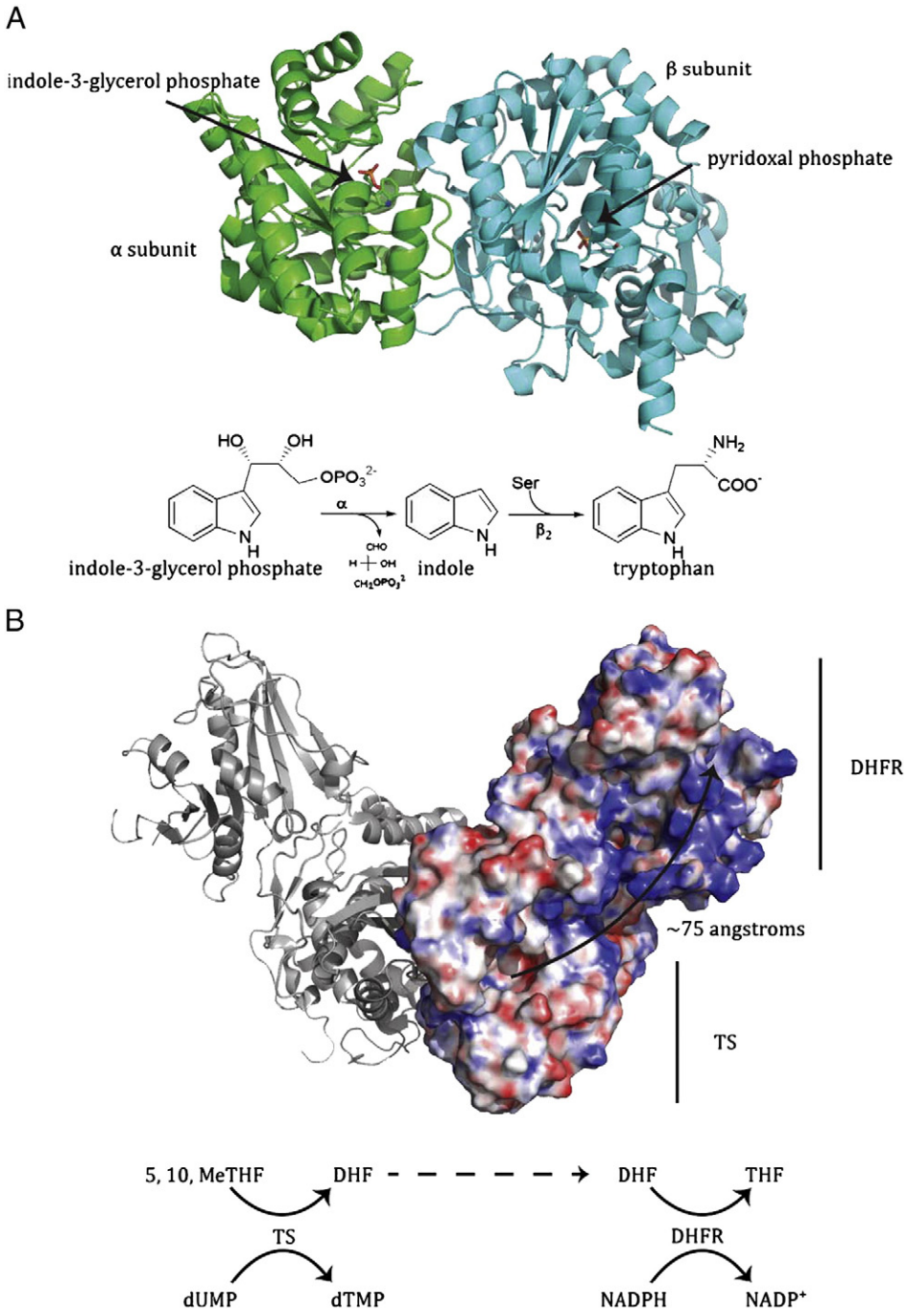

Fig. 1. A. Structure of the tryptophan synthase α–β dimer. Tryptophan synthetase is an $\alpha_2\beta_2$ tetramer; the α subunit catalyzes the formation of indole and glyceraldehyde-3-phosphate from indole-3-glycerol phosphate; the β subunit catalyzes the condensation of indole with serine to form tryptophan in a pyridoxal phosphate-dependent mechanism. The figure shows a model of the structure of the tryptophan synthase α–β dimer from *Salmonella* with bound indole-3-glycerol phosphate in the α subunit and pyridoxal phosphate in the β subunit (PDB ID: 1KFB). A 25 Å long hydrophobic channel is proposed to connect the two active sites that allows indole to move directly between the two subunits. Below is shown the reaction pathway for the synthesis of tryptophan from indole-3-glycerol phosphate. B. Structure of the bifunctional dihydrofolate reductase (DHFR)-thymidylate synthase (TS). The bi-functional enzyme catalyzes two sequential steps in the reaction pathway, converting 5,10-methylenetetrahydrofolate (5, 10, MeTHF) + dUMP to dihydrofolate (DHF) + dTMP in the first TS step, and dihydrofolate + NADPH + H^+ to tetrahydrofolate (THF) + $NADP^+$ in the second DHFR step. The figure shows the structure of the DHFR-TS protein from *Trypanosoma cruzi*. The protein crystallized as a dimer and a ribbon model is shown on the left and a surface charge model on the right (PDB ID: 3KJS). The active sites are 75 Å apart as indicated by the arrow and it is proposed that DHF is channeled from the active site of TS directly to the active site of DHFR along a surface "electrostatic highway".

mobility mediated by proton-coupled dipeptide uptake into intestinal enterocytes has been noted [38]. In this regard it may be useful to think of CO_2 as a "virtual" proton that can diffuse rapidly in cells as it would not be buffered by binding to cellular proteins and small molecules as would a positively-charged proton. CO_2 can be instantaneously hydrated to form H^+ and HCO_3^- by the action of CAII, an enzyme with a rapid turnover of ~10^6/s.

CAII also interacts with the Na^+/H^+ exchanger [39,40], suggesting a tight coupling between CAII and pH-regulatory transport systems. This is highlighted by *Nhe1*$^{-/-}$ knock-out mice that express higher levels of CAII with an increased abundance of an AE2–CAII complex and Cl^-/HCO_3^- activity in the parotid plasma membrane consistent with the presence of a bicarbonate transport metabolon [41]. Carbonic anhydrase activity and NHE1 are also required for renal sulfate secretion

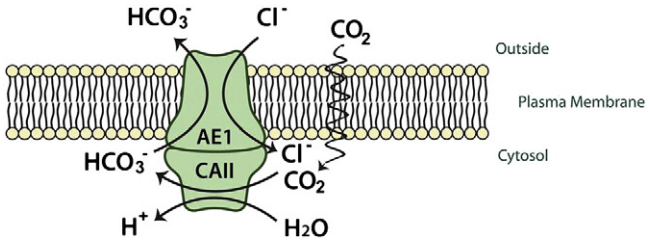

Fig. 2. A red cell bicarbonate transport metabolon. In the tissues like muscle, carbon dioxide (CO_2) that enters the red cell by diffusion (or perhaps via the aquaporin (AQP1) water channel) is hydrated by cytosolic carbonic anhydrase II (CAII) that is associated with the Band 3 anion exchanger 1 (AE1) membrane protein to produce a proton (H^+) and bicarbonate (HCO_3^-). The bicarbonate can be channeled directly to AE1 and exported in exchange for chloride. The proton is buffered by hemoglobin resulting in a decreased affinity for oxygen (Bohr effect). The net result of this bicarbonate transport metabolon is conversion of plasma carbon dioxide to bicarbonate and delivery of a proton to the cytosol. During gas exchange in the lungs, the system reverses. Bicarbonate that enters the red cell in exchange for chloride via AE1 is channeled to CAII, consuming a proton and producing H_2O and CO_2 that can diffuse out of the cell.

suggesting that CAII can be involved in more than carbon dioxide, bicarbonate or proton transport [42].

Some bacteria contain bicarbonate transporters fused directly to a β-type carbonic anhydrase, or encode bicarbonate transporters and carbonic anhydrases in the same operon [43], indicating the importance of this association throughout biology. For example, the *Pseudomonas* genome (www.pseudomonas.com) encodes four members of the SLC26 (SulP) anion transporter family (PA0029, 0103, 1647 and 2563). SLC26 anion transporters typically consist of an amino-terminal membrane domain with 10–12 TM segments and a carboxyl-terminal sulfate transporter and anti-sigma factor antagonist (STAS) domain [44]. PA0103 has a carbonic anhydrase (PA0102) in the same operon, suggesting that it is a bicarbonate transporter. There are three SLC26 family members in *Mycobacterium tuberculosis* (Rv1707, 1739 and 3273), one of which (Rv3273) contains a carbonic anhydrase fused to the C-terminus of the membrane domain rather than a STAS domain [43]. The Rv1739c protein mediates sulfate uptake when expressed in *Escherichia coli* but required the activity of the CysTWA ABC sulfate permease [45]. The structure of STAS domain of Rv1739c was determined and it binds guanine nucleotides, suggesting a regulatory role [46]. Cyanobacteria like *Synechococcus* carry out light-dependent CO_2 uptake that results in the net formation of bicarbonate mediated by a complex of NAD(P)H dehydrogenases (NDH) and Chp X and Y gene products that are likely carbonic anhydrases [47]. Inspired by the fused bacterial transporters, the Casey lab at the University of Alberta [48] joined CAII to the carboxyl-terminal tail of AE1 and found that although this chimera transported at lower rates than native AE1, it accelerated the rate of bicarbonate transport when compared with AE1 fused to an inactive CAII or when CA activity was blocked by an inhibitor. Thus, bicarbonate transport metabolons exist in a range of organisms from bacteria to humans and provide a mechanism that directly couples bicarbonate transport to the regulation of intracellular pH.

4. Glucose transport metabolons and glycolysis

Glucose that enters cells by facilitated diffusion through the glucose transporter (GLUT) family is phosphorylated by hexokinase or the low affinity glucokinase present in liver and certain other organs. This provides a sink for glucose within cells effectively preventing back flux of this important nutrient. The phosphorylation of glucose also provides immediate entry into the glycolytic or other pathways. This is an example of a membrane transport metabolon that links import of a substrate with an enzyme responsible for the first steps in its metabolism. There is evidence for an association of hexokinase with some members of the GLUT family of glucose transporters. In skeletal muscle, the expression of GLUT4 and hexokinase II is tightly coordinated at the transcriptional level [49]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) competes with hexokinase II for binding to GLUT4 [50]. Binding of hexokinase could facilitate glucose entry into glycolysis, while binding of GAPDH could inhibit this process, indicating reciprocal regulation of glucose transport.

Glycolytic enzymes exist as a complex—a metabolon, on the cytosolic surface of the plasma membrane of red blood cells by a direct physical interaction with Band 3, a very abundant integral membrane protein [8,51–53]. The ATP produced by glycolysis in the red cell, not "free" ATP, is used to fuel the Na^+/K^+ ATPase, the major energy consumer in the cell. Indeed, there is evidence that a "membrane-bound" pool of ATP exists and is linked to the ATPase [54,55]. Fig. 3 provides a model for a red cell membrane transport metabolon that uses a membrane-associated glycolytic complex to create a localized pool of metabolites (glucose, lactate, ATP and protons) that link various transport systems. Glucose that enters the red cell via the GLUT1 glucose transporter is metabolized via the membrane-bound glycolytic complex. The lactate produce by anaerobic respiration is removed for the red cell by the MCT-1 lactate transporter (SLC16A1) in a proton-dependent manner, thereby also removing protons produced in this process. The ATP produced can be used locally by ATP-driven pumps like the Na^+/K^+ ATPase. Glucose, lactate, nucleotides and protons may form a membrane-associated pool, sequestered by the transporters and enzymes that make up this membrane transport metabolon. Membrane proteins like those in the red cell can play a key role in the organization and control of cellular metabolism through an interaction with metabolic enzymes.

There is evidence that metabolites derived from exogenous sources do not mix with intracellular intermediates, consistent with channeling of intermediates in the glycolytic pathway [7]. In muscle, glycolytic metabolites generated from glucose do not mix with those generated from the breakdown of glycogen [56]. A similar finding was made in fibroblasts [57], suggesting that glycolytic enzymes form a complex sequestering glucose and its metabolites. This is also true in *E. coli* where in vivo isotope dilution experiments showed that fructose-1,6-bisphosphate is channeled by the glycolytic pathways efficiently to CO_2 [58]. Interestingly, studies of renal transport of glucose in dog kidney showed that glucose that enters the apical membrane of epithelial cells via the sodium glucose co-transporter (SGLT) family is not metabolized and exits intact into the blood

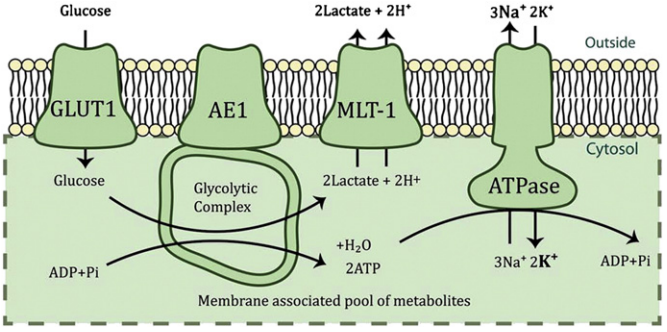

Fig. 3. A red cell glucose transport metabolon. This metabolon creates membrane-associated pools of metabolites indicated by the dotted rectangle that flow between and couple different transport systems. In this model, glucose enters the red cell via the GLUT1 glucose transporter to encounter a membrane-associated glycolytic complex bound to AE1, that consumes ADP and phosphate (Pi) to produce two ATPs, two lactate anions and two protons per glucose consumed. The ATP forms a membrane-associated pool that is used directly to fuel the Na^+/K^+ ATPase, regenerating ADP and phosphate that can be recycled back into glycolysis. The lactate is transported out of the red cell in a proton-dependent manner by the MCT-1 lactate transporter, clearing both the lactate and the protons. The net result of this metabolon is that extracellular glucose is converted to two extracellular lactates and two protons, while providing intracellular ATP used by the ATPase to pump sodium and potassium ions against their concentration gradients.

through the basolateral membrane (via GLUT transporters) [59,60]. This imported glucose does not mix with glucose that is formed within the cell by gluconeogenesis. There are invaginations of the basolateral membrane that are in close proximity to the apical membranes. These contacts could be "hot spots" for glucose transfer across the cell. Thus, there are organized pools of metabolites like glucose within cells and metabolons like the glycolytic complex play a role in sequestering and channeling metabolites.

Phosphofructokinase (PFK) has been reported to bind to the α4 subunit of the renal H^+ ATPase [61]. Mutations linked to the inherited disease, renal tubular acidosis that affect this interaction resulted in a decrease in proton transport. This membrane transporter may provide a binding site for glycolytic enzymes on the apical membrane in the acid secreting α-intercalated cells of the kidney. Perhaps the proton produced in the PFK reaction during the hydrolysis of ATP is transported directly out of the cell by the H^+ ATPase, an example of a membrane transport metabolon that directly couples an enzyme and a transporter.

5. Amino acid transport metabolons

In polarized epithelial cells amino acids are taken up across the apical membrane by members of the SLC7 cationic amino acid transporter/glycoprotein associated family [62]. In other regions of the intestine or nephron, the SLC15 proton-oligopeptide co-transport system predominates, taking up small peptides [63]. Peptides taken up on the apical side of polarized intestinal and kidney cells are rapidly hydrolyzed by intracellular peptidases to their constituent amino acids that are then transported into the blood by basolateral amino acid transporters. It may be worthwhile examining whether these peptidases associate with the transporters. Early efforts to clone amino acid transporters by functional expression in *Xenopus* oocytes identified rBAT and 4F2hc as the stimulating factor [64,65]. These single span type II membrane glycoprotein SLC3 family members form the heavy subunits of the heteromeric amino acid transporters and are covalently associated with the transport protein via a disulfide bond. It is interesting to note that type II aminopeptidase enzymes that are anchored to the apical surface of intestinal and kidney cells are associated with these amino acid uptake systems and stimulate the uptake of amino acids [66]. The amino acids released by aminopeptidases on the membrane surface may be channeled directly into associated amino acid transporters. It would be interesting to know if these enzymes are physically associated with the amino acid transport system, or if the heavy SLC3 subunits themselves are enzymes as suggested by sequence comparisons of rBAT with amylases [67].

The SLC1 family of amino acid transporters includes five high-affinity glutamate transporters (SLC1A1, A2, A3, A6 and A7) and two neutral amino acid transporters (SLC1A4 and A5) expressed in the brain and kidney [68]. The SLC1A1transporter (also known as EAAC1 and EAAT3) mediates the uptake of glutamate into neurons by the co-transport of 3 Na^+ and a H^+ in exchange for 1 K^+ providing a powerful driving force to reduce the synaptic levels of glutamate to the nM range. Interestingly, glutamine synthase co-localizes with SLC1A3 (EAAT1, GLAST) in retinal glia cells suggesting that the transporter and the enzyme work together to mediate the uptake and metabolism of synaptically-released glutamate to terminate the neurotransmitter function of glutamate [69]. Indeed knock-down of the glial SLC1A3 transporter results in an increased level of extracellular glutamate [70]. The SLC1 transporters consist of ~10 TM segments and a long hydrophobic C-terminal cytosolic tail. It would be interesting to determine whether glutamine synthase interacts directly with SLC1A3 and the role the C-terminal tail plays in this interaction.

A novel member of the SLC22 organic anion/cation transporter (OAT) family, OAT-PG mediates the uptake of prostaglandin E_2 (PGE_2) across the basolateral membrane of epithelial cells in proximal tubule of the kidney [71]. Interestingly, the inactivating enzyme 15-hydroxyprostaglandin dehydrogenase is also localized to the basolateral membrane converting PGE_2 to 15-keto-PGE_2, which is not a substrate for the transporter. The coupling of the transporter and the enzyme that metabolizes the substrate would facilitate uptake and clearance of PGE_2 from the blood, and particularly from the basolateral surface of the epithelial cells.

6. Mitochondrial transport metabolons

The electron transport chain present in the mitochondrial inner membrane and inner membrane of bacteria is a well-known example of a membrane transport metabolon, whereby electron flow is coupled directly to proton pumping. In bioenergetics there is also a linkage of transport and metabolism in complexes such as the F_1F_0ATPase, where a proton gradient is tightly coupled to ATP synthesis, linking proton transport and energy metabolism. P-type ATPases present in the plasma membrane use the energy of ATP to pump ions like calcium, sodium/potassium and protons against their concentration gradients, coupling an enzyme activity to transport within the same polypeptide [72].

Mitochondrial carriers encoded by the SLC25 gene family operate primarily as exchangers or proton-driven co-transporters to shuttle a variety of metabolites across the inner mitochondrial membrane [73]. The human genome encodes about ~75 mitochondrial transport proteins all related in structure to the ADP/ATP carrier and classified into 37 sub-families based on the transported substrate [74]. These membrane proteins typically consist of dimers. Each ~30 kDa subunit contains six TM segments and mediates transport.

The urea cycle is a metabolon spanning the mitochondrion and the cytosol linked by a transporter (Fig. 4) [75]. The ornithine carrier (ORC1) SLC25A15 carries out ornithine/citrulline exchange across the mitochondrial inner membrane and is the central link joining the cytosolic and mitochondrial components of the urea cycle. Ornithine produced by arginase in the cytosol is transported into rat liver mitochondria and is used preferentially by ornithine transcarbamoylase for citrulline synthesis [76]. Citrulline thus formed is exported from the mitochondria and channeled into urea synthesis in the cytosol [77], regenerating ornithine that is taken up in the next cycle. The mitochondrial components of the urea cycle are organized into a metabolon on the inner membrane and a similar organization of the enzyme components of the cycle located in the cytosol may also occur [75]. There is evidence [78] that carbamoyl phosphate synthetase and ornithine transcarbamoylase associate with the inner mitochondrial membrane.

The first five steps in arginine biosynthesis in yeast take place in the mitochondrial matrix beginning with imported glutamate and ending with ornithine that exits the mitochondrion via the ARG11 ornithine carrier. The first three enzymes of the arginine biosynthesis pathway in yeast (acetylglutamate synthase, acetylglutamate kinase and acetylglutamyl phosphate reductase) are associated within the mitochondrion to form a metabolon that converts glutamate into N-acetylglutamate-5-semialdehyde, which is converted in two more steps to ornithine that enters the urea cycle to form arginine [79,80]. An amino acid metabolon involving a dynamic interaction of the amino-transferase and the E1 decarboxylase component of the branched-chain α-keto acid dehydrogenase enzyme complex has been reported [81].

Within mitochondria, components of the Krebs cycle are also associated as a complex with the surface of the inner membrane [82]. Malate that is taken up by the SLC25A1 citrate/malate exchanger (Fig. 5) is converted to oxaloacetate by malate dehydrogenase and then to citrate by citrate synthase, the citrate exiting the mitochondria in exchange for malate. Citrate is used in the cytosol as a substrate for pathways involved in fatty acid and sterol biosynthesis. Computer modeling studies have shown that a complex of sequential mitochondrial enzymes allows channeling to occur along an electrostatic channel connecting malate dehydrogenase, citrate synthase

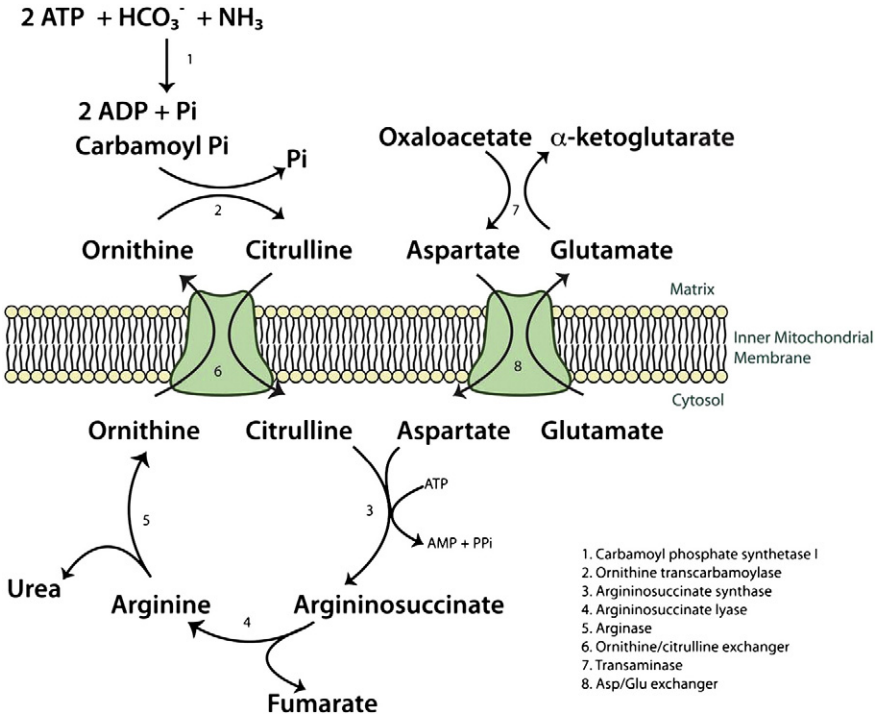

Fig. 4. A urea cycle metabolon. The enzymes that make up the urea cycle are compartmentalized in the cytosol and the mitochondrial matrix and are linked together by the ornithine/citrulline exchanger. The urea cycle consists of five enzymes: 1) carbamoyl phosphate synthetase I, 2) ornithine transcarbamoylase, 3) argininosuccinate synthetase, 4) argininosuccinate lyase and 5) arginase. The first two enzymes are located in the mitochondrial matrix and the latter three enzymes form a complex in the cytosol. The two parts of the urea cycle are linked together by 6) an ornithine/citrulline exchanger that transports these two substrates across the inner mitochondrial membrane. One of the amino groups in urea is derived by ammonia provided by cytosolic glutamate dehydrogenase (not shown) while the second amino group is derived from aspartate that is produced by a 7) transaminase reaction within the mitochondrion. The aspartate is transported into the cytosol in exchange for glutamate by 8) an aspartate/glutamate exchanger. Carbamoyl phosphate synthetase and ornithine transcarbamoylase are associated with the inner mitochondrial membrane, an interaction that could be mediated by the ornithine/citrulline exchanger.

and aconitase [83]. The association of this complex with the citrate/malate exchanger would create a membrane transport metabolon, channeling intermediates not only between sequential enzymes but also to and from the transporter.

7. Bacterial membrane transport metabolons

Do membrane transport metabolons exist in bacteria? We recently solved the crystal structure of a complex between the STAS domain of the YchM SLC26 anion transporter family member in *E. coli* with acyl carrier protein (ACP), linking bicarbonate transport and fatty acid biosynthesis [84]. Fig. 6 shows the structure of the complex of the STAS domain and ACP. The interaction is mediated in part by the prosthetic group of ACP, which in the complex consists of an activated malonyl group linked to phosphopantethionine. This indicates that the interaction of ACP with YchM depends on the metabolic flow into the fatty acid biosynthesis pathway and the level of malonyl ACP in the cell. Fatty acid biosynthesis is absolutely dependent upon bicarbonate [85], which is used in the first step to form malonyl-CoA from acetyl-CoA by the enzyme acetyl-CoA carboxylase. The malonyl group is then transferred to ACP in a second step by transferase. The malonyl-ACP is the activated donor for the subsequent elongation steps in fatty acid biosynthesis. YchM may channel bicarbarbonate taken up from the media directly into fatty acid biosynthesis (Fig. 6B). Alternatively, the interaction of the malonyl-ACP with YchM may be part of a sensor system allowing the bacterium to reduce the uptake of bicarbonate into the cell when the fatty acid biosynthesis pathway is fully charged as indicated by the level of malonyl-ACP. The complex of the STAS domain with ACP provides strong evidence for the existence of a membrane transport metabolon in *E. coli*, an interaction that was captured serendipitously in a study to solve the structure of the isolated STAS domain over-expressed in *E. coli*. The association of YchM with components of fatty acid metabolism was confirmed by genetic interactions, suggesting that sensitive high throughput methods are a valuable approach to detect interactions between membrane proteins and enzymes that could be followed up by structural studies.

7.1. Polycistronic operons in E. coli encoding transport proteins and enzymes

We present evidence for a genetic and a functional linkage between transporters and enzymes based on an analysis of operons in *E. coli* encoding polycistronic mRNAs that are coordinately translated into a transporter and one or more enzymes. The sequencing of bacterial genomes including *E. coli* [86] has provided a wealth of information (ECOCYC: http://ecocyc.org/, Regulon DB: http://regulondb.ccg.unam.mx/) concerning the organization of genes into operons [87]. In *E. coli*, genes encoding transport and binding proteins make up the largest well-defined functional group (427 genes) comprising about 10% of the genome [86]. However, relatively few high-resolution structures of transporters have been determined [88] although this is improving as indicated by the growing list of known membrane protein structures tabulated by the Stephen White laboratory at UC Irvine (http://blanco.biomol.uci.edt/mpstruc/listall/list). Of the 2584 predicted operons, 73% have only one gene, 17% two genes, 5% three genes and 6% with four or more genes. Genomics and proteomics studies of organisms like *E. coli* have identified specific protein interactions that form multisubunit protein complexes and elaborate cellular protein networks [1,4,89] or those proteins associated with the cell envelope [4,90].

Table 1 provides a summary of polycistronic operons in the *E. coli* encoding transport proteins and metabolic enzymes. The data in Table 1 were complied from a search of *E. coli* operons in RegulonDB (http://regulondb.ccg.unam.mx) and ECOCYC (http://ecocyc.org/) based on the original classification of *E. coli* gene products by Riley

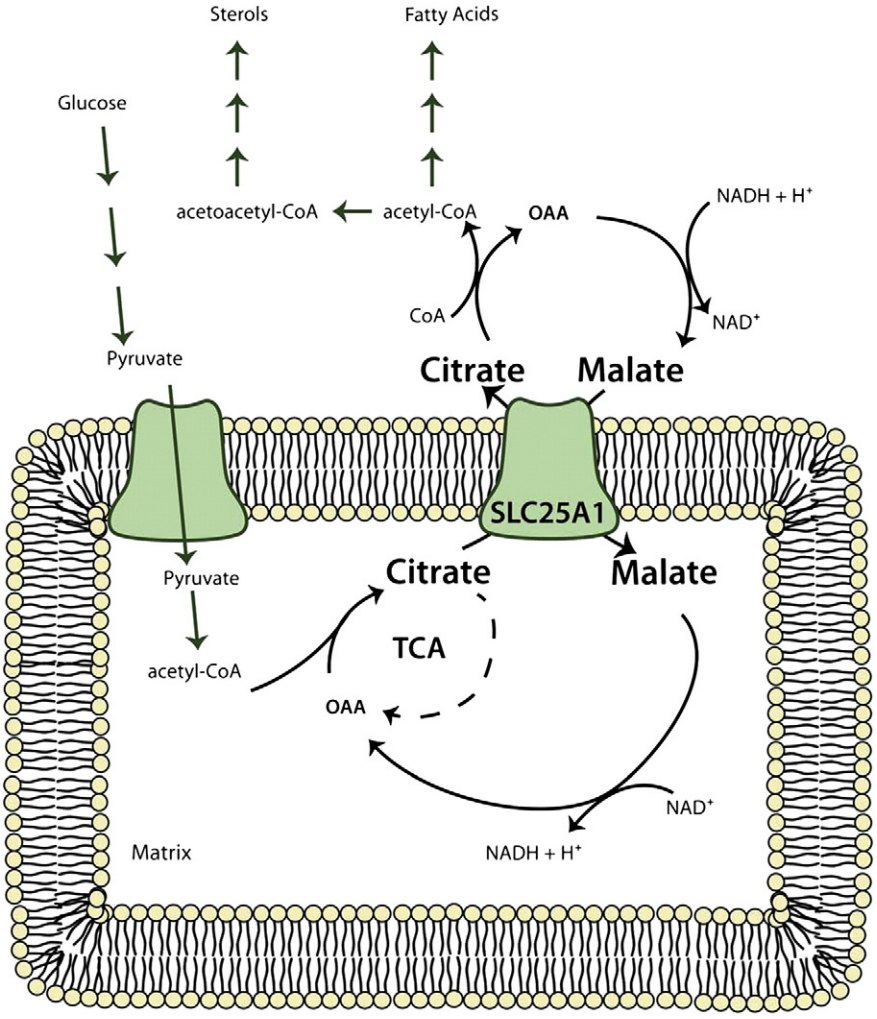

Fig. 5. A mitochondrial dicarboxylate transport metabolon. The enzymes that make up the Krebs (TCA) cycle are compartmentalized as a complex within the mitochondrion producing citrate by citrate synthetase from oxaloacetate and acetyl-coA. The acetyl-coA is derived from pyruvate that entered the mitochondrion through a pyruvate carrier. The SLC25A1 citrate carrier (ClC) provides citrate to the cytosol, which can be used by ATP-citrate lyase to produce oxaloacetate and acetyl-CoA that is essential for fatty acid and sterol biosynthesis. The oxaloacetate is reduced to malate by cytosolic malate dehydrogenase and can then re-enter the mitochondrion by citrate/malate exchange. In this case, SLC25A1 exchanging citrate and malate across the mitochondrial membrane connects two different metabolic pathways. Other members of the large SLC25 family (A1–27) play similar roles in shuttling metabolites across the mitochondrial inner membrane.

[91,92] using the search terms "transporter" or "transporter predicted" and "enzyme" or "enzyme predicted". A manual curation of all operons encoding polycistronic mRNAs listed in the *E. coli* Operon Table complied by the Bioinformatics Research Unit (bru.dna.affrc.go.jp/operons/ecoli/operons.html) that encode a transporter was also performed. Many of these operons encode a transporter and a single enzyme that can convert the transported substrate into product. In some cases one of the products is also a substrate for the transporter, which can be immediately effluxed through the same transporter in an exchange, or antiport mechanism. Other operons contain multiple enzymes that form a metabolic pathway to break down the transported substrate to produce energy for the cell or required substrates. Transporters are membrane proteins that are inserted co-translationally into the inner membrane of the bacterium. This targeting would localize the polycistronic mRNA to the membrane. Thus, the enzyme produced from the same mRNA would be localized to the vicinity of the membrane in close proximity to the transporter during its biosynthesis, thereby facilitating an interaction and formation of a functional complex.

The data in Table 1 clearly indicate that a genetic linkage exists between transport systems and enzymes in many *E. coli* polycistronic operons. As described below for each of these systems, there is also a functional linkage between uptake and metabolism as the enzymes are typically involved in the metabolism of the imported substrate. As shown in Fig. 7 a physical interaction of a transport and an enzyme would form a functional membrane transport metabolon, allowing the imported substrate to flow directly to the enzyme. The uptake of the substrate can be the rate-limiting step in metabolism, although certain enzymes in a pathway may also provide another point of metabolic regulation. Furthermore, the presence of transporters and enzymes within the same bacterial operon shows that transport and metabolism pathways have co-evolved and that the expression of the transporter and enzyme is co-regulated at the transcriptional and translational levels. Finally, the direct interaction of bacterial transporters and enzymes encoded within the same operon has not been extensively studied. Like metabolons, this may be because the interactions are very weak, resulting in enzyme dissociation when bacterial envelopes are prepared or when the membrane is solubilized in detergent solutions resulting in dissociation of the complex in the dilute solution. The characterization of complexes that may exist between transporters and enzymes is an area that requires further examination and the data summarized in Table 1 provides a guide to potential interactions that occur at the genetic and functional levels but may also exist at the structural level. The transport proteins whose structures have been solved to high

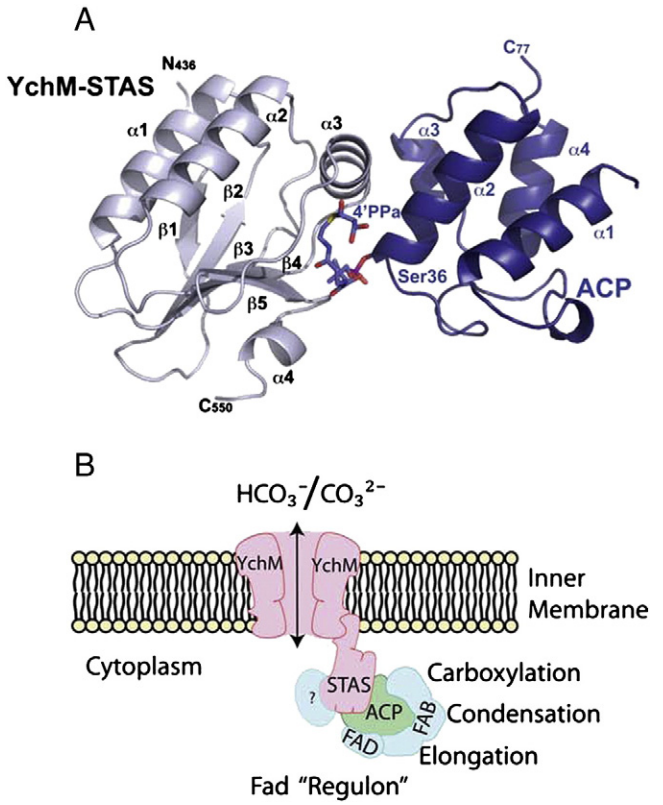

Fig. 6. A bacterial bicarbonate transport metabolon. A. A complex of the STAS domain from *E. coli* YchM and acyl carrier protein (ACP) determined by X-ray crystallography (PDB ID: 3NY7). B. A model for a bicarbonate transport metabolon in *E. coli* that links bicarbonate transport with fatty acid biosynthesis (FAB) and degradation (FAD). The STAS domain of YchM binds directly to ACP and that is part of an enzyme complex involved in the metabolism of fatty acids. In one model, bicarbonate that enters the cell via YchM is channeled directly into fatty acid synthesis. Bicarbonate is essential for fatty acid biosynthesis as a substrate for acetyl-CoA carboxylase. The malonyl group is then transferred to ACP where it is used in the condensation step. It is the malonyl-ACP intermediate that interacts with the STAS domain as shown in panel A.

resolution are limited and are highlighted in bold in Table 1. The known structures are illustrated in Fig. 8 along with the potential interacting protein drawn to scale. While these polycistronic operons suggest a tight functional and perhaps a structural linkage it is also possible that transporters and enzymes encoded in separate operons can also form functional complexes. These complexes may involve a direct interaction between the transporters and the enzymes or could be mediated by accessory proteins.

Genes within operons typically encode proteins that are linked functionally and they are subject to coordinated regulation. For example, the *trp* operon is repressed if the amino acid tryptophan is present in the media. Conversely, if *E. coli* is growing on lactose as its sole carbon source rather than glucose, the *lac* operon is turned on producing three products: Lac permease (lacY), a well-characterized membrane protein of known structure [93] that transports lactose across the inner bacterial membrane in a proton-dependent manner; β-galactosidase (lacZ) that hydolyzes lactose into glucose and galactose; and a transacetylase (lacA), whose role is unknown. The lac operon represents an example of a membrane transport metabolon, whereby a transport protein and an enzyme involved in the metabolism of the transported substrate are encoded in the same operon. It would be interesting to determine whether β-galactosidase can interact directly with lac permease and what effect this interaction has on uptake and flux into the metabolic pathway. The structures of lacY, lacA and lacZ are all known, so docking and molecular dynamic simulations could be carried out.

7.2. Two gene operons in E. coli encoding a transporter and a related enzyme

The following sections provide an analysis of polycistronic operons in *E. coli* that encode a transporter and an enzyme. Our analysis reveals that not only is there a genetic linkage between the transporter and enzyme indicating coordination of expression, but also that the substrate for the transport system and the enzyme are most often one and the same. Furthermore, the product of the enzyme is often also a substrate for the transporter allowing for import of a substrate, conversion to product, and efflux from the cell—the net result being conversion of extracellular substrate to product by a cytosolic enzyme. There is limited data available demonstrating a direct interaction between bacterial transporters and the enzymes metabolizing the imported compounds. One purpose of this review is to stimulate such studies.

7.2.1. Acid resistance transport metabolons

There are a number of operons in *E. coli* that contain only two genes encoding a transporter and an enzyme that operating together may form a simple membrane transport metabolon (Table 1). Several of these coupled transport and enzyme systems are involved in acid resistance in *E. coli*. The cad operon encodes two genes: an inner membrane lysine transporter (CadB) and a cytosolic lysine decarboxylase (CadA) that produces cadaverine. CadA is part of a lysine-dependent acid resistance mechanism induced under acidic conditions. CadA consumes a proton to convert lysine to cadaverine and CO_2 that can diffuse from the cell. The cadaverine is effluxed from the cell in exchange for lysine by CadB as in the model shown in Fig. 9A. Interestingly, CadB can operate in two modes: as a proton-lysine symporter or a lysine/cadaverine antiporter, taking up lysine in exchange for cadaverine produced by the decarboxylase as illustrated in Fig. 9A. We hypothesize that CadA interacts directly with CadB, channeling substrates between the enzyme and the transporter. In an antiporter mode the net result is conversion of extracellular lysine to cadaverine and cytosolic consumption of a proton and production of CO_2 that can exit the cell by diffusion providing protection against acid stress. CadA has been shown to interact with RavA, an AAA^+ ATPase, which may play a chaperonin-like role in the assembly of protein complexes [94].

Similarly, an ornithine transporter (PotE) and an ornithine decarboxylase (SpeF) are encoded in the same operon. The SpeF ornithine decarboxylase is one of two such enzymes in *E. coli* that consume a proton to convert ornithine to putrescine and CO_2. SpeF is inducible and is a defense mechanism against low pH, while SpeC is encoded in a different operon and is a constitutive biosynthetic form involved in putrescine production. PotE, like CadB can operate as a symporter or an antiporter. Although no evidence for a direct interaction of the decarboxylase with the transporter is currently available, such an interaction would allow for tight coupling of the two processes; the net result being conversion of extracellular ornithine to putrescine. Indeed, a physical interaction of carboxylases like SpeF with transporters like PotE may change the mode of transport from a proton-driven symporter (Fig. 9B) to an antiporter (Fig. 9A). A third acid-induced arginine decarboxylase (AdiA) is encoded in a single gene operon, which is located close to the operon encoding AdiC, the arginine/agmatine antiporter. AdiC is a homodimer with each subunit containing 12 TM segments that bind arginine by hydrogen bonds to the main chain atoms [95]. AdiA is an 800 kDa decamer (a pentamer of five homodimers) active under acidic conditions (optimal pH 5.2), while at neutral pH it dissociates into inactive homodimers [96]. AdiC and AdiA working together as a complex as illustrated in Fig. 9A would form an extreme acid resistance transport metabolon [97]. We are currently studying whether a pH dependent interaction between the AdiC arginine transporter and the AdiA arginine decarboxylase can be demonstrated using purified components and by computational docking experiments.

Table 1
E. coli polycistronic operons encoding transport proteins and enzymes.

Operon	Transported substrate	Transport protein[a]	Enzymes	Enzyme activity [citation][b]
abg	p-Aminobenzoyl glutamate	AbgT	AbgA/B	p-Aminobenzoyl glutamate hydrolase
			Ogt	Methyltransferase
acs, *act*	Acetate/glycolate	ActP	AcsA	Acetyl-CoA synthetase
			YjcH	Inner membrane protein
all, *gcl*, *glx*, *hyi*, *ybb*	Allantoin	YbbW/Y	AllB	Allantoinase
			Gcl	Glyoxylate carboligase
			GlxK	Glycerate kinase
			GlxR	Tartronate semialdehyde reductase
			Hyi	Hydroxypyruvate isomerase
amt	Ammonium/ammonia	**AmtB**	GlnK	Uridylylated regulatory protein [96][b]
ars	Arsenite	ArsB	ArsC	Arsenate reductase
			ArsR	Repressor
asp/*dcu*	Dicarboxylates	DcuA	AspA	Aspartase
cad	Lysine	CadB	CadA	Lysine decarboxylase
cai	Carnitine	**CaiT**	CaiA	Carnitine oxidoreductase
			Cai B	Carnitine dehydrase
			CaiC	Carnitine CoA ligase
			CaiD	Carnitine racemase
			Cai E	Unknown
cod	Cytosine	CodB	CodA	Cytosine deaminase
cyn	Cyanate	CynX	CynS	Cyanate aminohydrolase
			CynT	Carbonic anhydrase
dcu/*fum*	Dicarboxylates	DcuB	FumB	Fumarase
dgo	Galactonate	DgoT	DgoA	2-Oxo-3-deoxygalactonate-6-phosphate aldolase
			DgoD	Galactonate dehydratase
			DgoK	2-Dehydro-3-deoxygalactonate kinase
			DgoR	Transcription regulator
dsd	Serine	DsdX	DsdA	Serine deaminase
foc/*pfl*	Formate	**FocA**	PflB	Pyruvate formate lyase
frl	Fructoselysine	FrlA	FrlB	Fructoselysine 6-phosphate deglycase
			FrlC	Fructoselysine 3-epimerase
			FrlD	Fructoselysine 6-kinase
			FrlR	Transcriptional regulator
fuc	Fucose	FucP	FucK	L-fuculokinase
			FucI	L-fucose kinase
			FucU	Unknown
			FucR	Regulator of fuc operon
gab	Amino-butyrate	GabP	GabD	Succinate semialdehyde DH
			GabT	Amino-butyrate aminotransferase
			CsiD	Carbon starvation induced gene
			LhgO	Hydroxyglutarate oxidase
gad	Glutamate	GadC	GadB	Glutamate decarboxylase
gar	Glucarate	GarP	GarK	Glycerate kinase
			GarL	α-Dehydro-β-deoxy-D-glucarate aldolase
			GarR	Tartronate semialdehyde reductase
			RnpB	RNase P
glc	Glycolate	GlcA	GlcB	Malate synthase
			GlcD	Glycolate oxidase
			GlcE	Glycolate oxidase
			GlcF	Glycolate oxidase
			GlcG	Unknown
glp	Glycerol-3-phosphate	**GlpT**	GlpQ	Glycerophosphodiester diesterase (periplasmic)
glp	Glycerol	**GlpF**	GlpK	Glycerol kinase [93][b]
			GlpX	Fructose 1,6-bisphosphatase
gnt	Gluconate	GntU	GntK	Gluconokinase
			GntR	Transcription repressor
gud	Glucarate	GudP	GudD	Glucarate dehydratase
			GudX	Unknown
lac	Lactose	**LacY**	LacZ	Beta-D-galactosidase
			LacA	Galactoside acetyltransferase
idn	Idonate	IdnT	IdnD	Idonate 5-dehydrogenase
			IdnO	5-Ketogluconate reductase
			IdnR	Transcription regulator
lld	Lactate	LldP	LldD	Lactate dehydrogenase
			LldR	Regulator of lld operon
mel	Melibiose	MelB	MelA	Alpha-galactosidase
nad/*pnu*	Nicotinamide mononucleotide	PnuC	NadA	Quinolinate synthase
nan	N-Acetyl neuraminic acid	NanT	NanA	NAN lyase
			NanE	*N*-Acetylmannosamine-6-phosphate epimerase
			NanK	*N*-Acetylmannosamine kinase
			YhcH	
nir	Nitrate	NirC	NirB/D	Nitrite reductase
			CysG	Uroporphryrin III C-methyltransferase

(continued on next page)

Table 1 (*continued*)

Operon	Transported substrate	Transport protein[a]	Enzymes	Enzyme activity [citation][b]
nup	Nucleosides	NupG	MltC	Murein transglycosylase
			MutY	Adenine transglycosylase
			YggX	Oxidation inhibitor
pot/spe	Ornithine	PotE	SpeF	Ornithine decarboxylase
psd/yje	Mechano-sensitive channel	YjeP	PsdA	Phosphatidyl serine decarboxylase
rha	L-rhamnose	RhaD	RhaB	L-Rhamnose kinase
			RhaA	L-Rhamnose isomerase
rut	Uracil	RutG	RutA	Pyrimidine oxygenase
			RutB	Ureidoacrylate amido hydrolase
			RutC	Aminoacrylate peracid reductase
			RutD	Aminoacrylate hydrolase
			RutE	Malonic semialdehyde reductase
			RutF	Flavin reductase
sda	Serine	SdaC	SdaB	Serine deaminase
tdc	Threonine	TdcC	TdcB	Threonine dehydratase
			TdcA	Regulator of tdc operon
			TdcD	Propionate kinase
			TdcE	Pyruvate formate
			TdcF	Ribonuclease
			TdcG	Serine dehydratase
teh	Tellurite	TehA	TehB	Tellurite methyltransferase
tna	Tryptophan	TnaB	TnaA	Tryptophanase
			TnaC	Leader peptide/attenuation
trk	K^+	TrkH	YigZ	Elongation factor
			PepQ	Proline dipeptidase
			HemG	Protoporphyrinogen oxidase
ttd	Tartrate	TtdT	TtdA/B	Tartrate dehydratase
uid	Glucuronides	UidB	UidA	Glucuronidase
			UidC	Outer membrane protein
ura	Uracil	**UraA**	Upp	Uracil phosphoribosyltransferase
xap	Xanthosine	XapB	XapA	Xanthosine phosphorylase

[a] The crystal structures of transporters indicated in bold have been determined.
[b] Cited reference provides evidence for an interaction between the enzyme and the transporter.

The gad operon is also part of an acid resistance system. GadC is glutamate/γ-aminobutyrate (GABA) antiporter. GadB is a glutamate decarboxylase that consumes a proton to convert glutamate to 4-aminobutyrate and CO_2. At neutral pH the 330 kDa GadB hexamer is inactive in the cytosol [98]. However at acidic pH it is activated exposing a short triple helix bundle and is bound to the membrane, perhaps through an interaction with GadC. This provides an example of how the interaction between a transporter and an enzyme may be dynamic allowing for regulation of the interaction and the flux through the system.

7.2.2. Dicarboxylate transport metabolons

The dcu/asp operon encodes DcuA, one of three transporters responsible for the uptake of C4 dicarboxylates like malate, succinate, fumarate, and the amino acid aspartate under anaerobic conditions. DcuA mediates dicarboxylate exchange in an antiport mechanism (Fig. 10A). AspA is an aspartase suggesting that one mode of action of this transport metabolon is that the aspartate that enters the cell via DcuA is converted to fumarate (and ammonia plus a proton). The same fumarate just formed is then transported out of the cell in exchange for aspartate, leaving ammonium inside the cell. A second C4 dicarboxylate transporter, DcuB is located in an operon that also encodes FumB, a fumerase that interconverts fumarate and malate, again suggesting that fumarate that enters the cell via DcuB is converted to malate directly by FumB, and malate then exits the cell via the same transporter in an antiport mechanism. DcuC is encoded by a single gene operon and carries out fumarate/succinate exchange effluxing succinate from cells under anaerobic conditions [99]. While exchange is favored, all three transporters (DcuA, B, C) can also mediate proton dependent uptake of C4 dicarboxylates in a symport mechanism (Fig. 10B). The association of the enzyme could promote the antiport mechanism and dissociation, the symport mechanism. It is possible that the transporters mediate anion/hydroxyl exchange rather than proton/anion co-transport, although most bacterial transport systems are driven by the proton gradient generated by the electron transport chain.

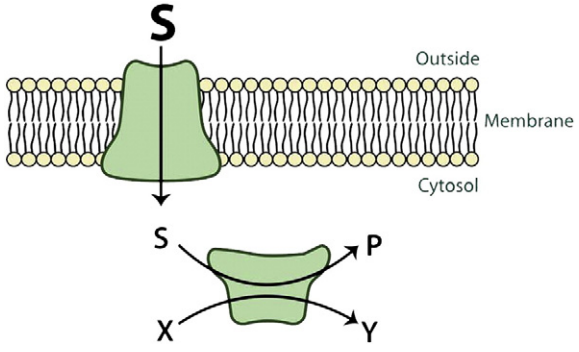

Fig. 7. A general model for membrane transport metabolon. The substrate (S) for the transport protein is transported across the membrane by facilitated transport down its gradient and channeled to an associated enzyme that produces a product (P). The coupling of the transporter and the enzyme helps drive the uptake process forward by mass action and allows the substrate to directly enter a metabolic pathway.

7.2.3. Amino acid transport metabolons

The serine transport system (sdaC) is linked to serine deaminase II (sdaB) that produces pyruvate for energy production and ammonia. Serine deaminase I (sdaA) is a single gene operon, while serine deaminase III (tdcG) is part of a large operon induced under anaerobic conditions that encodes the threonine transporter tdcC.

The tna operon encodes the tryptophan transporter, TnaB and TnaA, a tryptophanase that catalyzes the hydrolysis of tryptophan to indole, pyruvate and ammonia allowing *E. coli* to grow on tryptophan as its sole carbon and nitrogen source. TnaC encodes a leader peptide involved in an attenuation mechanism resulting in ribosome stalling and translation arrest in the absence of tryptophan that prevents transcription of tnaA and B. *E. coli* contains two other tryptophan transport systems, AroP and Mtr. AroP is a general aromatic amino

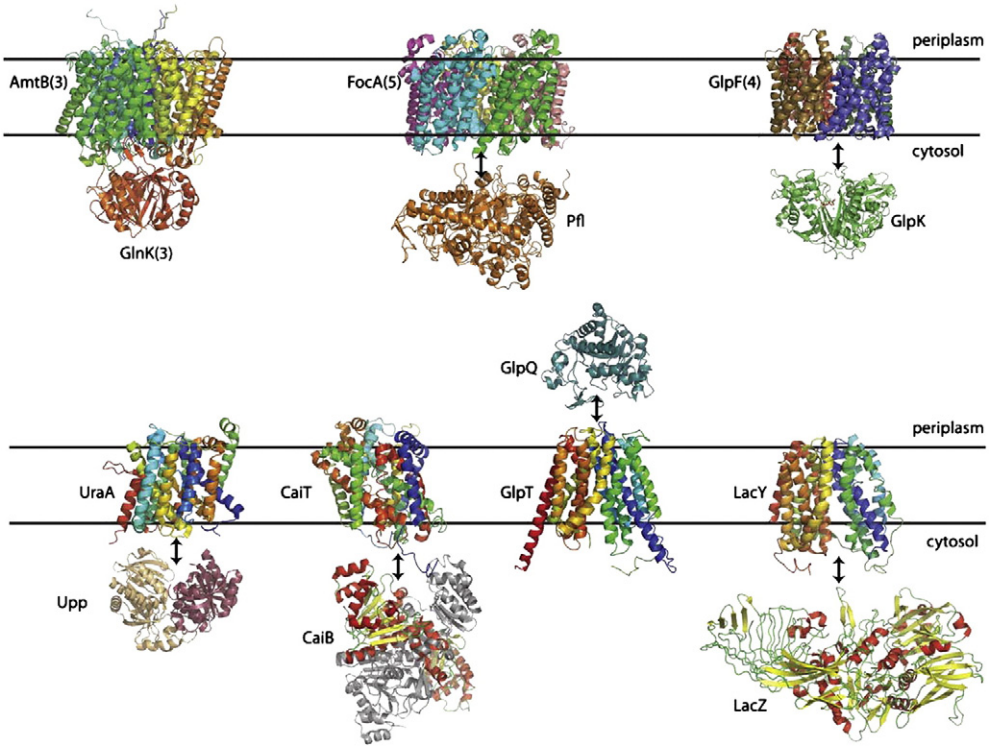

Fig. 8. A gallery of crystal structures of *E. coli* transport proteins and the enzymes that metabolize the imported substrates. These transport proteins and enzymes are encoded in the same operon; the enzyme metabolizes the imported substrate; suggesting that they may interact allowing channeling between the transporter and the enzyme. Note, the example of a true complex is the co-crystal structure of trimeric AmtB bound to regulator GlnK (PDB ID: 2NUU) shown in the top row. Binding of GlnK to AmtB is inhibitory to ammonia transport. The other structural representatives of potential *E. coli* transporter and enzyme complexes shown are: Row 1: pentameric FocA (PDB ID: 3KCU) and pyruvate formate-lyase (2PFL-monomer); tetrameric GlpF (PDB ID: 1FX8) and GlpK (PDB ID: 1BWF), also known as glycerol kinase (GK). Row 2: UraA (PDB ID: 3QE7) with dimeric Upp (PDB ID: 1T0U); CaiT (PDB ID: 2WSW) with dimeric CaiB (PDB ID: 1XK6), CaiA/C/DE are not shown; GlpT (PDB ID: 1PW4) with monomeric periplasmic GlpQ (PDB ID: 1T8Q); LacY (PDB ID: 2CFQ) with a monomer of LacZ (PDB ID: 1F4H). LacA is not shown. All models are drawn to scale.

acid transporter that can take up tryptophan, tyrosine and phenylalanine in a proton-driven manner with a low Km of 1 μM. Mtr is a high affinity transporter for tryptophan and indole and is homologous to TnaB. Expression of the *mtr* gene is repressed by the Trp repressor in the presence of tryptophan. Tryptophan that enters the cell via the Mtr or AroP transporters if left intact could then be used for protein biosynthesis while tryptophan that enters via the Tna system is broken down by the coupled action of a transporter and enzyme to provide carbon and nitrogen sources for cell growth. Thus, not every tryptophan that enters the cell has the same fate, again showing that the membrane transport metabolons play a role in substrate channeling.

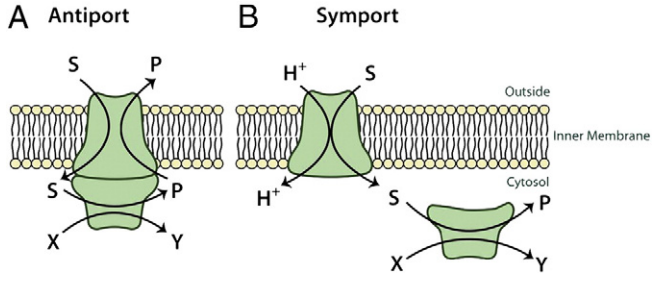

Fig. 9. Bacterial membrane transport metabolons working in antiport or symport modes. A. A bacterial membrane metabolon working in antiport mode. The substrate (S) imported by the transporter is channeled to an associated enzyme producing a product (P) that is exported in an antiport or exchange mechanism. The net result is conversion of extracellular S to P. The enzyme also converts a co-substrate X to product Y in the cytosol. In the case of the acid response systems in *E. coli*, the reaction consumes a cytosolic proton and produces CO_2 that can diffuse out of the cell. B. A bacterial membrane metabolon working in symport mode. The substrate (S) for the transporter is co-transported into the cell along with a proton. The substrate is converted to product (P) by an enzyme allowing P to be released into the cytosol. The net result is the uptake of the substrate and conversion to product within the cell. A single transporter may operate in either antiport or symport mode depending on whether the enzyme is tightly coupled to the transporter or not.

7.2.4. Nucleic acid base transport metabolon

CodB is a proton-cytosine symporter. CodA is a cytosine deaminase that produces adenine for pyrimidine biosynthesis, and ammonia. Transcription of the cod operon, which encodes both CodA and CodB is induced under nitrogen-limiting conditions suggesting that the role of this coupled system is to supply the bacterium with ammonia for biosynthetic purposes. In this case, CodB is predicted to act as a cytosine/adenine antiporter.

There are two uracil transport systems in *E. coli* encoded by two different operons that include enzymes. UraA is a proton-uracil symporter and the structure of this transporter has been determined [100]. It contains 14 TM segments divided into two inverted repeats with a pair of anti-parallel β-strands between TM segments 3 and 10 involved in substrate recognition. Also encoded in the operon is Upp, uracil phosphoribosyltransferase, which catalyzes the conversion of uracil and 5-phospho-D-ribose-1-diphosphate into uridine-5-phosphate and pyrophosphate. Uracil imported into the cell could be channeled directly into the formation of the nucleotide. The second uptake system for uracil (rut) contains a complex set of enzymes. RutG is a proton-uracil symporter that imports pyrimidine bases to be used as a nitrogen source beginning with RutA, a pyrimidine oxygenase, through the RutB, C, D, E and F enzymes to produce 3-hydroxypropionate, ammonia and carbamate that decomposes to form a second ammonia and carbon dioxide allowing *E. coli* to use imported pyrimidines as their sole source of nitrogen. So, in one system uracil is channeled into pyrimidine biosynthesis while in the

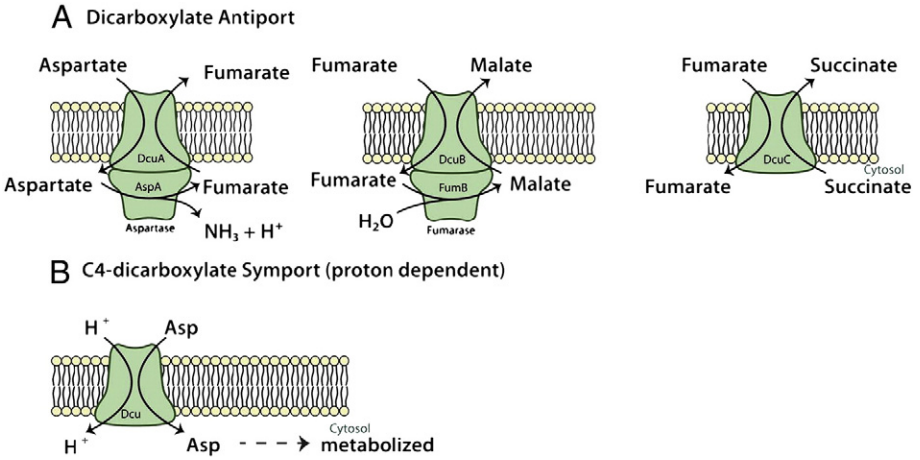

Fig. 10. Dicarboxylate transport metabolons. A. The DcuA dicarboxylate transporter imports aspartate in exchange for fumarate. An associated AspA aspartase converts aspartate to fumarate, which is transported out of the cell. Similarly the DcuB transporter imports fumarate in exchange for malate. The fumarate is converted to malate by an associated FumB fumarase. The DcuC fumarate/succinate exchanger is encoded in a single gene operon. It may also be associated with an enzyme involved in the further metabolism of fumarate and succinate. B. A dicarboxylate transporter working in symport mode to drive the uptake and subsequent metabolism of the substrate. In this case the product of the enzyme reaction remains in the cytosol to be further metabolized.

other it provides a source of nitrogen. Clearly, every uracil imported into *E. coli* does not meet the same fate, highlighting the importance of membrane transport metabolons in sequestering pools of substrates, often for very different metabolic purposes. It would be interesting to determine whether RutA-F exists as a complex and whether this complex interacts directly with the RutG transporter.

7.2.5. Gluconate transport metabolons

The gnt operon contains three genes: GntU—one of four gluconate transporters in *E. coli*, GntK, a gluconate kinase that produces 6-phosphogluconate and GntR, a transcription repressor. GntU can operate as a proton-gluconate symporter, importing gluconate that is converted to 6-phospho-gluconate by the GntK kinase, the first step in the gluconate degradative pathway. GntT, GntP and IdnT are homologous to GntU. IdnT is also a gluconante transporter encoded in an operon that also encodes two enzymes: IdaD, an idonate 5-dehydrogenase, IdaO, a 5-ketogluconate reductase and IdaR, a transcription regulator. IdaO catalyzes the NAD(P) dependent conversion of imported gluconate to 5-ketogluconate, which is then reduced to idonate using NAD(P)H by IdnD. IdnT can also transport idonate and likely operates as an idonate/gluconate exchanger. If idonate is imported it can be converted to gluconate by the combined action of IdaD and IdaO, which can then be effluxed by IdaD in an exchange reaction. The net result is the interconversion of extracellular idonate and gluconate. If IdnT acts in a proton symport mechanism, then the imported gluconate or idonate can be interconverted in the cytosol. GntP and GntT are both encoded by monocistronic operons and likely function as proton-gluconate symporters. DsdX is a serine-specific permease that is related to the Gnt family of gluconate transporters. DsdA is a serine deaminase that produces pyruvate and ammonium. Based on this information, DsdX is likely a serine/pyruvate antiporter that may associate with DsdA (Fig. 7).

7.2.6. Glycerol transport metabolons

There are examples of bacterial transport metabolons where the enzyme produces the substrate for the transporter. GlpT is a member of the major facilitator superfamily that transports glycerol-3-phosphate into the cytoplasm in exchange for phosphate and its structure has been determined [101]. The glp operon contains GlpT, the permease and GlpQ, a periplasmic glycerophosphodiester phosphodiesterase. This enzyme hydrolyzes deacylated phospholipids to an alcohol and glycerol-3-phosphate that is subsequently transported into the cell. In this case, the periplasmic enzyme is responsible for forming the transported substrate (Fig. 8). The possibility of a direct association of GlpQ with GlpT should be examined. In contrast, the cytosolic phosphodiesterase UgpQ encoded in ugp ABC transporter operon (Table 3) has been reported [102] to only hydrolyze glycerophosphodiesters transported by the ugp system and not cytosolic compounds, a good example of substrate channeling from a transporter to an enzyme.

Glycerol kinase catalyzes the rate-limiting step in glycerol utilization. The glycerol kinase gene (glpK) is in the same operon as the glycerol transporter GlpF. The structure of GlpF is known [103] and it is a member of the aquaporin (AQP) family of water channels (Fig. 8). An interaction between these two proteins has been reported [104] to stimulate the kinase activity of GlpK providing an important regulatory mechanism. The kinase would act to trap glycerol inside the cell. Glycerol 3-phosphate is converted into dihydroxylacetone phosphate and then glyceraldehyde-3-phosphate to join the gluconeogenesis pathway. The operon also encodes GlpX, a type II fructose 1,6-bis-phosphatase, an enzyme involved in gluconeogenesis during growth on glycerol to produce fructose-6-phosphate and phosphate [105,106]. Transcription of GlpX is inducible by glycerol and glycerol-3-phosphate. Deletion of GlpX is not lethal as the main fructose 1,6-bis-phosphatase in *E. coli* is Fbp, a type 1 enzyme [107]. A fructose 1,6-bis-phosphatase, YggF related to GlpX (58% sequence identity), is encoded in a large *cmt/ygg* PTS operon (Table 3) and is involved in mannitol uptake and metabolism.

7.2.7. Ammonia transport metabolons

AmtB is responsible for the uptake of ammonia/ammonium necessary for growth under limiting nitrogen conditions. The AmtB crystal structure has been determined to high resolution by the Winkler and Stroud laboratories [108,109]. AmtB is a native trimer; each monomer consisting of 11 transmembrane segments with each half spanning the membrane with opposite polarity forming a 20 Å "gas" channel. The AmtB ammonium/ammonia transporter is encoded by an operon that also encodes GlnK, a uridylylated regulatory protein that interacts with the carboxyl-terminal region of AmtB forming an ammonium sensing system [110]. In the presence of high ammonium (ammonium shock) the intracellular level of glutamine rises. This leads to deuridylylation of GlnK due to inhibition of the uridylyltransferase activity of GlnD and stimulation of its deuridylylation activity by glutamine. The deuridylylated GlnK binds to AmtB inhibiting ammonium transport, thereby limiting ammonia uptake. The structure of this complex is shown in Fig. 8.

7.2.8. Formate transport metabolon

The crystal structure of the FocA formate transporter has been solved to 2.25 Å [111]. FocA forms a pentameric aquaporin-like structure that forms a central hourglass shaped pore. Each subunit contains six TM segments with the first three helices related by a quasi-2-fold axis in the plane of the membrane that form a channel through the membrane. Encoded in the same operon is PflB, a pyruvate-formate lyase that converts pyruvate to acetyl-CoA and formate. The formate leaves the cell via FocA to be metabolized by the formate dehydrogenases, as FDH-N and FDH-O that have their active sites facing the periplasmic space. In this case an association of the pyruvate-formate lyase with the cytosolic side of FocA could convert imported pyruvate into formate, which could exit via FocA in an antiport mechanism. Without associated pyruvate-formate lyase FocA could operate as a channel or symporter. FocA is related to NirC, a nitrite transporter that is encoded in the same operon that encodes NirB/D a nitrite reductase, which uses NAD(P)H to form ammonium. Both NarK and U are nitrate/nitrite exchangers encoded in monocistronic operons. A separate operon narZYWV encodes proteins necessary to assemble and form the membrane-bound nitrate reductase.

7.2.9. A mechano-sensitive channel "regulon"

YjeP is a putative potassium-dependent mechano-sensitive channel homologous to KefA/MscK. Interestingly, the operon encoding YjeP also encodes psd, a phosphatidylserine decarboxylase that produces phosphatidylethanolamine. Thus, there may be a link between the lipid composition of the membrane surrounding the channel and mechanosensing. Lipid composition can affect the lateral pressure exerted on intrinsic membrane proteins, which in turn can affect the protein's conformation, association and function [112]. Molecular dynamics simulations of lipid bilayers have shown that phosphatidylcholine exerts a larger lateral pressure in the headgroup region but a lower lateral pressure in the central hydrophobic core than phosphatidylethanolamine, which may affect the gating of mechano-sensitive channels [113]. Changes in the lateral pressure exerted on a membrane protein like YjeP from the lipid bilayer may occur by conversion of boundary lipids with an anionic headgroup like phosphatidylserine to those with a smaller, zwitterionic headgroup like phosphatidylethanolamine. In this case, the associated enzyme modifies the lipid environment around the channel, perhaps regulating its opening and closing.

7.2.10. Two gene operons encoding metal transport metabolons

There are other examples of two gene operons in *E. coli* that encode a transporter and an enzyme that form a functional complex. The teh operon encodes TehA, a tellurite transporter and tehB, a tellurite S-adenosylmethionine-dependent methyl transferase (Table 1). Working together they confer tellurite resistance. Similarly, the ars operon encodes ArsB, an arsenate/proton antiporter and ArsC, an arsenate reductase that uses glutathione to produce arsenite, in addition to a repressor (ArsR) that is sensitive to arsenite (Table 1). In this case arsenite produced within the cell by the reductase is effluxed out in exchange for a proton.

8. Polycistronic bacterial operons encoding transport proteins and multiple enzymes

8.1. Cai operon, carnitine transport and metabolism

Some operons in *E. coli* encode a transporter and multiple enzymes that form a metabolic pathway (Table 1). The cai operons encode CaiT a carnitine transporter, while the cai A, B, C, D and E gene products are involved in the metabolism of carnitine beginning with CiaC, which transfers CoA to carnitine as a first degradation step that leads to cronontobetainylCoA. The structure of the homotrimeric CaiT protein has been determined to 3.15 Å resolution [114], with each subunit containing 12 transmembrane segments and four bound carnitine molecules that outline the translocation pathway (Fig. 8).

8.2. The dgo operon, galactonate transport and metabolism

The dgo operon encodes 5 genes, DgoT being a galactonate transporter. DgoD is a D-galactonate dehydratase that catalyzes the first step in the degradation of D-galactonate, dehydration to form 2-dehydro-3-deoxygalactonate, which is then phosphorylated by DgoK, a kinase. The 2-oxo-3-deoxygalactonate 6-phosphate is then converted to glyceraldehyde-3-phosphate and pyruvate by an aldolase, DgoA, and these two compounds enter the glycolytic pathway. Thus an entire degradative pathway is linked to a transporter importing the raw material. It would be worthwhile exploring whether the DgoD/K/A proteins form a complex that associates directly with DgoT.

8.3. The gab operon, γ-amino butyrate transport and metabolism

GabP imports 4-aminobutyrate that when combined with 2-oxoglutarate is converted to glutamate and succinate semi-aldehyde by the transaminase GabT. Succinate semi-aldehyde is then converted to succinate by the GabD dehydrogenase. The 2-oxoglutarate is produced from 2-hydroxyglutarate by LhgO, an L-2-hydroxyglutarate oxidase. CsiD has an unknown function.

8.4. The gar operons, glutarate transport and metabolism

GarL is an aldolase that breaks 5-dehydro-4-deoxy-D-glutarate, the product of glutarate dehydratase, into pyruvate and tartronate semi-aldehyde, which is reduced by GarR to form glycerate. GarK is a kinase that forms 2-phosphoglycerate from glycerate. GarP is an uncharacterized member of the major facilitator superfamily (MFS) group of transporters that based on sequence similarity may function as a proton-driven glutarate symporter and perhaps a glutarate/glycerate antiporter.

8.5. Other polycistronic operons and metabolism

GlcD, E and F are subunits of a glycolate oxidase that converts glycolate imported by GlcA into glyoxylate, which is in turn converted to malate by GlcB, one of two malate synthases in *E. coli*.

NanA is an N-acetylneuraminic acid lyase that catalyzes the first step in the degradation of N-acetylneuraminic acid (sialic acid), imported by NanT, producing N-acetylmannosamine and acetyl-CoA. NanK is an N-acetylmannosamine kinase, whose product N-acetylmannosamine-6-phosphate is broken down by NanE epimerase into N-acetylglucosamine-6-phosphate.

RutA catalyzes the ring opening of uracil imported by the RutG transporter, the first step in a pyrimidine degradative pathway encoded by the polycistronic rutA-G operon.

The *tdc* operon encodes 7 proteins (TdcA-G), TdcC being a threonine transporter. TdcB is a threonine dehydrase that catalyzes the first step in threonine degradation. These examples indicate that enzyme complexes that form the entire metabolic pathways may associate with transport systems that import the raw materials.

9. ABC transporter operons encoding enzymes

9.1. The ugp operons and glycerol-3-phosphate transport and metabolism

E. coli contains a second glycerol-3-phosphate transport system encoded by the *ugp* operon. This operon encodes a typical ABC transporter consisting of a periplasmic binding protein (ugpB), two membrane proteins (ugpE and C) that together form the transport unit and an ATPase dimer ($ugpA_2$) that provides the energy for transport (Fig. 11). This operon also encodes a cytosolic enzyme

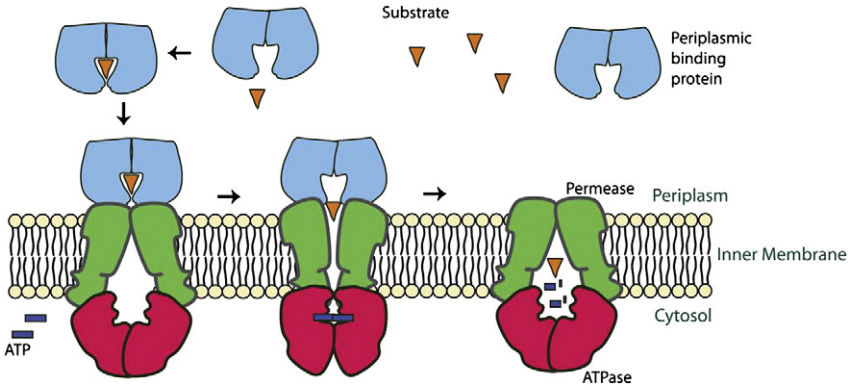

Fig. 11. A model of bacterial ABC transporters that consists of a periplasmic binding protein (blue), dimeric transmembrane subunits (green) that may be identical and a cytosolic ATPase dimer (red). The substrate (orange triangle) binds first to a specific periplasmic binding protein, which then docks onto the outward-facing permease releasing the substrate into the channel. An ATP-driven conformational change in the permease puts it into the inward facing conformation that opens the substrate-binding site to the cytosol with subsequent hydrolysis of ATP.

(ugpQ), a glycerophosphodiester diesterase that hydrolyzes diesters to produce glycerol-3-phosphate and the corresponding alcohol. Glycerophosphodiesters are hydrolyzed by UgpQ only when transported by the Ugp system, while cytosolic glycerophosphodiesters are not substrates for this enzyme [102]. This indicates a tight coupling of glycerol-3-phosphate uptake and its metabolism, suggesting that UgpQ may interact directly with other components of this uptake system. The ugp operon is under the control of the pho regulon (see below) and is induced under low phosphate conditions.

9.2. The cys operons and cysteine transport and metabolism

There are other examples of operons for ABC transporters that also contain a gene for a metabolic enzyme (Table 2). The cys operon encodes a periplasmic thiosulfate binding protein (CysA), a pair of transport proteins (CysW and U), and an ATPase dimer (CysA) [115], the typical four protein arrangement for bacterial ABC transporters. In addition, the operon encodes CysM, which is cysteine synthase B, an O-acetylserine sulfhydrylase. Cysteine synthase B catalyzes the formation of cysteine from sulfide and O-acetylserine and can use thiosulfate in place of hydrogen sulfide to produce S-sulfocysteine [116].

Two other *cys* operons are involved in sulfate assimilation and cysteine biosynthesis. The first operon (*cys*D, *cys*N, *cys*C) encodes CysD and N that form a sulfate adenylyltransferase converting sulfate and ATP to adenosine-5′-phosphosulfate, which is a substrate for CysC, an adenylylsulfate kinase producing phosphoadenosine-5′-phosphosulfate. The second operon (cysH, cysI, cysJ) produces hydrogen sulfide through two reductase steps. The hydrogen sulfide then combines with O-acetyl-serine to form cysteine in a step catalyzed by cysteine synthases (CysM and CysK). O-acetyl serine is formed from serine and acetyl-CoA in a step catalyzed by serine acetyltransferase, CysE, which is encoded in its own operon. Unlike cysteine synthase B, cysteine synthase A (cysK) forms a complex with serine acetyltransferase (cysE).

In plants, cysteine synthetase binds to the C-terminal STAS domain of the Sultr1;2 sulfate transporter, a member of the SLC26 family of anion transporters [117]. The sulfate ion is transported into plant root cells, reduced to sulfide, which is then bonded to O-acetylserine through the activity of cysteine synthase to form cysteine, the first organic molecule of the sulfate assimilation pathway.

9.3. The phn operons and phosphonate/phosphate transport and metabolism

The *E. coli* phosphate (*pho*) regulon includes over 30 genes arranged in nine separate operons: *eda*, *phnCDEFGHIJKLMNOP*, *phoA-psiF*, *phoBR*, *phoE*, *phoH*, *psiE*, *pstSCAB-phoU*, and *ugpBAECQ* [106]. The 14-gene *phnCDEFGHIJKLMNOP* operon is involved in phosphonate uptake and metabolism. PhnD is the periplasmic binding protein, PhnC is the ATPase and PhnE is the membrane transporter. Proteins PhnG-M make up a membrane-associated C-P lyase responsible for phosphonate biodegradation with subunits G, H, I, J, K forming a multi-subunit complex [118]. C-P lyase activity does not require PhnCDE and occurs on the outer surface of the inner membrane.

PstS, PstC, PstA and PstB, encoded in the *pstSCAB-phoU* operon, form a high affinity ABC phosphate transporter. PstS is the periplasmic phosphate binding protein, Pst B is the ATPase and PstA and C form the membrane transporter. The repressor PhoR, a phosphate sensor kinase that phosphorylates the transcription factor PhoB, associates with the PstSCABU complex. Under limiting phosphate, PhoR is in an active signaling conformation resulting in the phosphorylation and activation of PhoB and subsequent induction of the Pho regulon. When phosphate levels are high the Pho system is repressed. Phosphate can be taken up by the constitutive low-affinity phosphate transporter, PitA. A third phosphate uptake system PitB, is repressed through the pho regulon when phosphate levels are low. The Pst transport system is induced when phosphate levels are low. PhoE is an outer membrane β-barrel porin that allows phosphate entry into the periplasmic space, while phoA is a periplasmic alkaline phosphatase. *psi*E and *psi*F are phosphate-starvation inducible genes.

As described above, the ugp operon (ugp*BAECQ*) encodes an ABC glycerol-3-phosphate uptake system (Table 2). UgpC is the ATP-binding protein, UgpA and UgpE are the membrane components, UgpB is the periplasmic binding protein and UgpQ is a cytosolic glycerophosphodiester phosphodiesterase [102,119]. Transcription of the *ugp* operon is under the control of the *pho* regulon. This system takes up glycerol-3-phosphate to provide essential phosphate under phosphate-limiting growth conditions. The *pho* regulon illustrates how the activity of genes encoded in different operons can be coordinately regulated to respond to changes in the environmental levels of essential nutrients like phosphate.

10. Phosphotransferase transport system

The bacterial phosphoenolpyruvate (PEP):glucose phosphotransferase (PTS) is an example of a bacterial membrane transport metabolon that was discovered by Saul Roseman in 1964 [120] and widely studied by his lab and others ever since [121]. *E. coli* can grow on certain fermentable carbon sources such as glucose, mannose, fructose, hexitols like mannitol and glucitol/sorbitol, hexosamines and N-acetylhexosamines like N-acetylglucosamine that are taken up by the PTS transport system, resulting in repression of other sugar uptake systems and enzymes. The sugars transported into the cell are phosphorylated during the transport process, favoring

Table 2
ABC Transporter Operons Encoding Enzymes.

Operon	Substrate	Periplasmic binding protein	Nucleotide-binding protein	Membrane subunits	Enzyme(s)
als	Allose	AlsB	AlsA	AlsC	AlsE Allulose-6-phosphate 3-epimerase RpiR Transcription regulator Lrp Transcription regulator RpiB Allose-6-phosphate/ribose-5-phosphate isomerase
btuCDE btuF	Vitamin B12	BtuF	BtuD	BtuC	BtuE Glutathione peroxidase Pfs 5′methylthioadenosine nucleosidase YadS Inner membrane protein
cys	Sulfate and thiosulfate	CysP and Sbp	$CysA_2$	CystT/W	CysM Cysteine synthase B
gsi	Glutathione	GsiB	$GsiA_2$	GsiC/D	IaaA Arginase III
lsr	Autoinducer A1–2	LsrB	LsrA	LsrC/D	LsrF Aldolase LsrG P-DPD degradation Tam Trans-aconitate methyltransferase
phn	Alkyl phosphonate	PhnD	PhnC	PhnE	PhnF Transcription regulator PhnO Acyltransferase PhnN Ribose 1,5 bisphosphokinase PhnG, H, I, J, K, L, M Carbon–phosphorus lyase complex PhnP Lyase accessory protein
rbs	Ribose	RbsB	RbsA	RbsC/D	RbsK Ribokinase RbsR DNA-binding regulatory
ssu	Aliphatic sulfonates	SsuA	$SsuB_2$	$SsuC_2$	SsuD $FMNH_2$-dep monooxygenase SsuE NAD(P)H-dep FMN reductase
tau	Taurine	TauA	$TauC_2$	$TauB_2$	Tau D Taurine dioxygenase
ugp	Glycerol-3-phosphate	UgpB	$UgpC_2$	UgpA/E	UgpQ Glycerophosphodiester phosphodiesterase
yec	?		YecC	YecS	DcyD Cysteine desulfhydrase

uptake. The phosphoryl group on phosphoenolpyruvate (PEP), a high-energy donor, is transferred initially to a histidine on enzyme E1 and then His-15 on HPr. Next, the phosphoryl group is transferred to the sugar-specific EII complex consisting of EIIA (EIII), EIIB and the transmembrane EIIC (Fig. 12). E1 and Hpr (PtsH) are common to all PTS systems and are encoded within the pts operon. The EII complex can consist of a single polypeptide (Class 1); two polypeptides (Class 2); or in the case of mannose PTS, three separate polypeptides (Class 3). There are two phosphorylation sites in the EII complexes of all three classes: two sites in the single polypeptide of Class 1; one site in each of the Class 2 proteins and two sites in each of the two domains that comprise $EIII^{Man}$, the soluble component of three polypeptides in this complex. The presence of components of PTS system such as the EII components within a single polypeptide demonstrates a close physical association of the components that may also exist between components that are separate polypeptides. Fig. 12 illustrates the crystal structures of the components of the bacterial PTS system.

The crystal structure of *E. coli* EI [122,123] was obtained by using Mg^{2+} and PEP to phosphorylate EI and then adding oxalate, a pyruvate analog. Each subunit of the EI dimer consists of three domains: PEP binds to the C-terminal domain, an α/β barrel fold binds PEP; the N-terminal domain, an α-helix bundle, binds HPr and the central domain, tethered to the N-terminal domain by two closely associated linkers and to the C-terminal domain by a long α-helix, contains the phosphorylatable histidine residue (His189).

The crystal structure of *E. coli* mannitol phosphotransferase IIA^{mtl} has been solved to 1.8 Å [124]. The protein consists of 5 β-strands flanked by 5 α-helices with the phosphorylated histidine (His65) located in a shallow crevice at the end of strand 3, where HPr is presumed to bind to accept the activated phosphate. The structures of HPr and $EIIA^{glc}$ from *E. coli* have been solved using NMR and X-ray diffraction [125]. The 10 kDa Hpr protein consists of a 4-stranded β-sheet covered on one side by one short and two long helices. The phosphorylated His 15 is located on a turn between the 4th strand and the 1st short helix. The $EIIA^{glc}$ from *E. coli* forms an anti-parallel β-sandwich backed by two short helical segments. The active site contains two face-to-face histidine residues, His 90 being the acceptor from HPr. In the presence of PTS sugars, $EIIA^{glc}$ its unphosphorylated state can form a complex with glycerol kinase, inhibiting the enzyme [126].

The first crystal structure of an EIIC membrane protein, that from *Bacillus cereus*, which transports diacetylchitobiose was determined

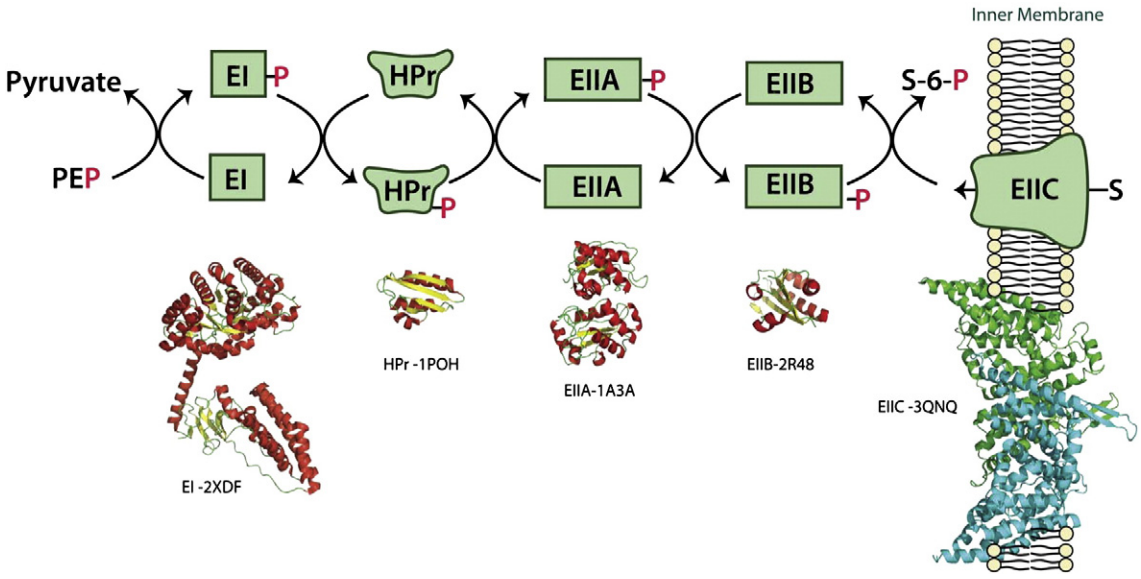

Fig. 12. A model for bacterial phosphotransferase system (PTS) that consists of EI, HPr, EIIABC shown here as distinct proteins (Class 3). The phosphoryl group donated by phosphoenolpyruvate is transferred first to EI, then to HPr, onto EIIA and B and finally to the sugar substrate during the translocation process by EIIC. Shown below are the known structures for the various components of the PTS machinery: E1 (*E. coli*—PDB ID: 2XDF); HPr (*E. coli*—PDB ID: 1POH); EIIA (*E. coli*—PDB ID: 1A3A); EIIB (Bacillus subtilis-PDB ID: 2R48) and EIIC (*Bacillus cereus*—PDB ID: 3QNQ) that transports diacetylchitobiose. Note that PDB ID: 2XDF shows the NMR structure of a complex of E1 and HPr.

in 2011 [127] by a group of investigators led by Ming Zhou and affiliated with the New York Structural Biology Center. EIIC is a homodimer, with a large interface between the amino-terminal halves of the two monomers. The carboxyl-terminal half of each monomer provides a large binding site for diacetylchitobiose, which occludes the sugar from both sides of the membrane with its site of phosphorylation near conserved His and Glu residues. Binding of enzymes to the cytosolic surface of the protein may unblock the transport pathway by dislocating the loop between TM helices 4 and 5 that forms the intracellular gate.

Some PTS operons encode enzymes that are involved in the further metabolism of the phosphorylated sugars (Table 3). These enzymes may be arranged as a complex that associates with the membrane via the PTS transport system forming a membrane transport metabolon. A simple PTS operon is the mng operon that encodes two proteins, MngA and MngB. MngA is a Class 3 EII protein responsible for the uptake and phosphorylation of 2-*O*-α-mannosyl-D-glycerate (MG) producing 2-O-(6-phospho-α-mannosyl)-D-glycerate during transit, which is then metabolized to mannose-6-phosphate and glycerate by MngB, an alpha-mannosidase.

Operons like asc, blg and tre also encode hydrolases that break down the imported phosphorylated disaccharides into a phosphorylated and non-phosphorylated monosaccharide, while other operons like fru encode kinases that add additional phosphate groups onto the imported sugars. FruF converts imported frucose-1-phosphate to fructose-1,6-bisphosphate, which then can enter the glycolytic pathway allowing *E. coli* to grow on fructose as a sole carbon source. Interestingly, FruB encodes a chimera of EII and HPr. These examples show that operons that encode proteins that associate to form a functional PTS transport system also encode enzymes that metabolize the imported substrate. It would be worthwhile examining whether the encoded enzymes can directly interact with the PTS complexes.

There are also some PTS operons that encode a multitude of metabolic enzymes. In some *E. coli* strains the aga operon encodes genes (*agaZVWEFASYBCDI*) responsible for the uptake and metabolism of D-galactosamine and *N*-acetyl-D-galactosamine (GalNAc). AgaB is enzyme IIB^{Gam}, AgaC is enzyme IIC^{Gam}, AgaD is enzyme IID^{Gam} and AgaF is enzyme $IIA^{Aga/Gam}$. The *aga* operon (*agaZVWASYBCDI*) also encodes enzymes for the metabolism of D-galactosamine and GalNAc; namely AgaA, a GalNAc-6-phosphate deacetylase, AgaS, a putative isomerase, AgaYZ, a tagatose-bisphosphate aldolase and AgaI, a GalNAc-6-phosphate deaminase providing an example of a complex membrane transport metabolon. These enzymes may themselves associate to form a metabolon that then interacts with the membrane component of the PTS system but this has yet to be examined. Furthermore, the dual transcription regulator Mlc (for Makes large colonies) controls the expression of PTS genes involved in the uptake of glucose. Mlc also interacts directly with active $EIICB^{Glc}$, sequestering Mlc. The structure of the Mlc–$EIIB^{Glc}$ complex has been solved to high resolution [128] showing that regulatory protein can interact directly with membrane transporters.

11. Yeast transport metabolons

In yeast there is tight genetic control of the expression of membrane proteins and enzymes that may form membrane transport metabolons, an example being the regulation of the *GAL* genes. These genes are repressed in the presence of glucose by the action of the transcription factor Mig1p. Galactose is taken up by the galactose permease Gal2p, one of about 20 hexose permeases in yeast. *Saccharomyces cerevisiae* converts galactose to glucose-6-phosphate using the five sequential enzymes of the Leloir pathway [129]: galactose mutarotase (Gal10p), galactokinase (Gal1p), galactose-1-phophate uridyltransferase (Gal7p) and UDP-galactose-4-epimerase (Gal10p). The next enzyme in the pathway, phosphoglucomutase (Pgm1p/2p), an O-glycosylated protein, controls the flux through the system [130]. In most organisms the enzymes are expressed as separate polypeptides. In yeast the mutarotase and the epimerase exist as a single polypeptide encoded by *GAL10*. The structure of Gal10p [131] reveals two fused domains connected by a short Type II turn: the amino-terminal epimerase domain and the carboxyl-terminal mutarotase domain. This fusion of first and the last enzymes in the pathway suggests that the four enzymes of the Leloir pathway form a metabolon that channels intermediates from one active site to the next thereby preventing the diffusion of essential or even toxic (galactose-1-phosphate) intermediates free into the cytosol [132]. The binding of the "Leloir" complex to the yeast membrane via interaction with Gal2p would create a galactose transport metabolon. It would certainly be worthwhile to continue to study the genetic, functional and now, in the context of membrane transport metabolons, the physical

Table 3
Phosphotransferase transport system (PTS, PEP group translocation) operons encoding metabolic enzymes.

Operon Class[a]	Substrate[b]	Enzyme EIIA (EIII)	Enzyme EIIB	Enzyme IIC/D	Enzyme (s)	Activity
agaZVWEFASYBCDI	N-acetyl-galactosamine	AgaF (deleted in K12)	AgaB/V	AgaC/D	AgaYZ	Tagatose-1-6-bisphosphate
Class 3	Aga, GalN, GalNAc, Gam			AgaW/E	AgaA	aldolase
					AgaS	GalNAc-6-phosphate deacetylase
					AgaI	Putative isomerase
						GalNAc-6-phosphate deaminase
asc	β-Glucosides	(IIA^{Glc})	AscF		AscB	Phospho-β-glucosidase
Class 2			IIB–IIC			
bgl	β-Glucosides	BglF			BglG	Regulatory protein
Class 1	Bgl, Glc, αMG, Cel	IIB–IIC–IIA			BglA/B	Phospho-β-glucosidase
chb	Chitobiose	ChbA	ChbB	ChbC	ChbF	Diacetylchitobiose-6-phosphate hydrolase
Class 3					ChbG	Unknown
					Chb R	Transcription regulator
cmt	Mannitol?	CmtB	CmtA		YggF	Fructose-1,6-bisphosphatase
Class 2			IIB–IIC		YggG	Predicted dehydrogenase
					YggC	Predicted nucleotide hydrolase
					YggD	Transcription regulator
dha	Dihydroxyacetone	DhaM		?	DhaK/L/M	Dihydroxyacetone kinase
Class 2		~Hpr~E1–EIIA–EIIB			DhaR	Transcription regulator
fru	Fructose	FruB	FruA		FruF(K)	1-Phosphofructokinase
Class 2	Fru, Sor, Glc, Man, Xtl	EIIA–M–HPr	IB′–IIB–IIC			
fry	Fructose	FryA	FryB	FryC	YfdE	Peptidase
Class 3	Peptides?	HPr–E1–EIIA			YpdF	Aminopeptidase
gut	Glucitol	GutB/SrlB	GutE	GutA	GutD	Sorbitol-6-phosphate dehydrogenase
Class 3	Gut, dArl, Gat, Mtl, Arl, Fru	(IIA)	IIB–IIC2	IIC1	GutQ	Arabinose-5-phosphate isomerase
					GutM/R	Transcription regulators
man	Mannose	ManX		Man Y/Z	None	
Class 3	Man, Glc, GlcNAc, GlcN, 2DG, αMg, Fru, Tre	IIA–IIB				
mng	2-O-α-Mannosyl-D-glycerate	MngA			MngB	Alpha-mannosidase
Class 1		EIIA–IIB–IIC				
mtl	Mannitol	MltA			MtlD	Mannitol-1-phosphate dehydrogenase
Class 1	Mtl, Gut, Arl, dArl	IIC–IIB–IIA			MtlR	Transcription regulator
mur	N-Ac-muraminic acid	(IIA^{Glc})	MurP		MurQ	N-Ac-muraminic acid 6-phosphate etherase
Class 2			EIIB–IIC		WfeW	Carboxypeptidase
nagE	N-Acglucosamine	NagE			None	N/A
Class 1	GlcNAc, MeGlcNAc	IIC–IIB–IIA				
nagBACD	Works together with NagE				Nag A	N-Acglucosamine deacetylase
					NagB	Glucosamine phosphate deaminase
					NagC	Co-repressor
					NagD	Putative phosphatase
ptsA	Fructose	PtsA	?	?	FsaB	Fructose-6-phosphate aldolase
Class 2/3		EI–EIIA			GldA	Glycerol dehydrogenase
ptsG	Glucose	(ccr)	ptsG		None	N/A
Class 2	Glc, αMG, 5-TG, GlcN, Sor, Man, 2DG	(IIA^{glc})	IIC–IIB			
scr	Sucrose	?	ScrA		ScrB	Sucrose-6-phosphate hydrolase
Class 2			EIIB–C			
tre	Trehalose	(IIA^{glc})	TreB		TreC	Trehalose-6-phosphate hydrolase
Class 2			EIIB–C			
ula	Ascorbate	UlaC	UlaB	UlaA	UlaD	3-Keto-L-gulonate 6-phosphate decarboxylase
Class 3					UlaE	L-Xylulose 5-phosphate 3-epimerase
					UlaF	L-Ribulose 5-phosphate 4-epimerase

Class 1, components IIA (III), IIB and IIC/D encoded in 1 polypeptide; class 2, components IIA, IIB and IIC/D encoded in 2 polypeptides and class 3, components IIA, IIB and IIC/D encoded in 3 or more polypeptides.

[a] The EII complexes consist of a single polypeptide (Class 1); two polypeptides (Class 2); or in the case of mannose PTS, three separate polypeptides (Class 3).

[b] Abbreviations: αMG, methyl α-glucoside; Arl, arabinitol; Bgal, β-galactoside; Bgl, β-glucosides (arbutin, salicin); Cel, cellobiose; dArl, 2-deoxyarabinohexitol; 2DG, 2-deoxyglucose; Dha, dihydroxyacetone; Fru, fructose; Gal, galactose; Gat, galactitol; Glc, glucose; GlcN, glucosamine; GlcNAc, N-acetylglucosamine; Gly, glycerol; Gnd, gluconate; Gut, glutitol; Lac; lactose; Mal, maltose; Man, mannose; MeGlcNAc, methyl α-N-acetylglucosaminide; mel, melibiose; Mtl, mannitol; Scr, sucrose; Sor, L-sorbose; 5TG, 5-thioglucose; Tre, trehalose; Xtl, xylitol.

interaction of yeast transporters and the enzymes that metabolize the imported substrates.

12. Conclusions

Membrane transport metabolons involve the genetic, functional and physical association of membrane transporters and cytosolic enzymes. Such an interaction allows substrates taken up by cells to be channeled directly into metabolic pathways. The immediate modification of the transported substrates would prevent back-flux through transporters, trapping the substrate inside the cell. Many of these facilitated transporters have a low affinity for their substrates, allowing the cell to respond to the level of nutrient in its environment with substrates entering the cell at a rate sensitive to their external concentration. The chemical modification of the transported substrate would ensure that an inward-directed gradient exists for the substrate. Furthermore, the association of an enzyme with a transporter may allow regulation of the rate of flux into cells. This may occur by mass action, with the enzyme providing a continuous down-hill gradient for the substrate, particularly important for facilitated diffusion mechanisms. In some cases the product of the enzyme reaction is also a substrate for the same transporter creating a tightly coupled

antiport mechanism. The binding of the enzymes could also affect the transport activity by an allosteric mechanism or the interaction with the transporter may affect the enzyme activity. Binding could be regulated by mechanisms such as phosphorylation or other modifications that affect protein interactions providing a means to regulate uptake based on metabolic demand. Evidence for a direct interaction of enzymes and transport proteins is sparse and is an area in the structural biology of membrane proteins that needs further attention. The data compiled in this review on polycistronic operons in *E. coli* that are known to encode transporters and enzymes is designed to provide a guide to such future studies on bacterial transport metabolons. It is important to determine the structural basis of the predicted interactions between transporters and enzymes to show whether they can indeed form a physical complex and to determine the effect of this interaction on their mutual activities and flux through functionally linked transport and metabolic systems.

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