# Unraveling structural and functional features of secondary transport proteins

Heuberger, Esther Helena Maria Laurentius

*Publication date:*
2001

# General introduction

## The relevance of membrane protein research

Biological membranes play an essential role in the existence of all living cells. They are composed of lipids and proteins, of which the lipids are mostly arranged in bilayer structures. Membranes form a barrier between the cell interior and its environment, which is not only crucial for the generation and interconversion of metabolic energy but also for the structural organization of the cell. Embedded in these membranes are the proteins that effect essential reactions such as the generation of metabolic energy, the import and export of various solutes and proteins, the transduction of signals, cell mobility and others. Statistical analysis of all known genome sequences predicts that 20 to 30% of all open reading frames encode integral membrane proteins (164).

Bacteria, eukarya and archaea have evolved a variety of mechanisms by which solutes can be transported. According to their energy coupling mechanisms, transport proteins can be divided into three distinct groups, being (Fig. 1): (A) primary transporters, which couple transport of a solute to a primary source of energy such as ATP or light; (B) secondary transporters, which use electrochemical gradients to drive transport; and (C) groups translocaters, which couple the transport of a solute to the chemical modification of the substrate. These different classes of transporters have in common that a transport cycle involves distinct conformational states of the protein, and the substrate is translocated in a stoichiometric relation with, for example, the ions that are co- or counter-transported or the ATP that is hydrolyzed. Distinct from these transport proteins are the channels (D), which do not use

metabolic energy, but rather allow passage of molecules down the concentration gradient in a non-stoichiometric process. Channels open and close in response to specific signals (ligands, voltage, mechanic stimulus) and in the open-state the translocation may involve $10^5$ to $10^9$ molecules per second, which is orders of magnitude higher than the turnover of the fastest primary or secondary transport system.

In the last decade, the analysis of the structure and function of membrane transport proteins gained enormous interest as many human diseases turned out to be associated with defects in transport, e.g. malfunctioning of the
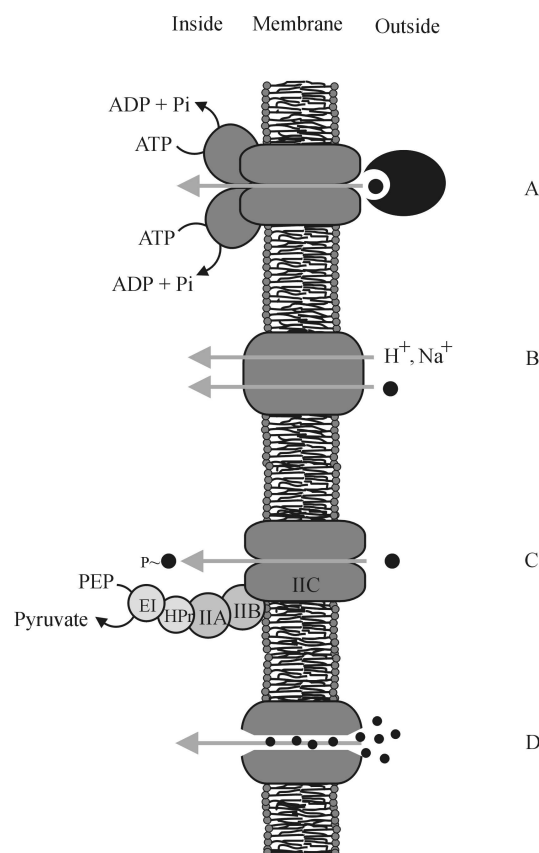


**Fig. 1. Different classes of solute transporters.** (A) Primary transporters; (B) Secondary transporters; (C) Groups translocaters; (D) Channel proteins.

glutamate and serotonine transporters in the nervous system can lead to neurological and psychiatric disorders, and defects in the catalysis or regulation of glucose transport across the renal brush-border membrane is the cause of diabetes. In addition, membrane proteins are involved in processes like drug-resistance and drug-targeting and they can be widely exploited for biotechnological purposes. So far, high resolution data is available for only a few transmembrane proteins, and of the systems involved in solute translocation, the structural information is restricted to channel proteins. To get a better insight in the functional and structural features of secondary transport proteins, the XylP protein from *Lactobacillus pentosus* and the LacS protein from *Streptococcus thermophilus* were studied comparatively. Both proteins belong to the galactoside-pentoside-hexuronide family, but differ structurally from each other as the LacS protein possesses an additional regulatory domain.

## Secondary transport proteins

### *General aspects.*

Secondary transporters use the free energy stored in the electrochemical gradient of protons, sodium ions or other solutes across the membrane to drive a uniport, symport or antiport reaction. The majority of the transporters are comprised of 10 to 14 predicted transmembrane (TM) α-helices, which is generally proposed to be the structural and functional unit for secondary transporters (91,108). Some proteins consist of only 6 transmembrane segments, like the members of the Mitochondrial Carrier (MC) Family (77), and even smaller proteins have been found in the Small Multidrug Resistance (SMR) Family. The drug antiporter EmrE from *Escherichia coli* belongs to the SMR family and is composed of only 4 TM α-helical segments. Sequence similarity between the first and the last half of 12 TM α-helical transporters suggests that these proteins have evolved from a gene duplication

of a six-α-helical polypeptide precursor. The two times 6 TM α-helical domains are generally connected via a large cytoplasmic loop. In some cases, this cytoplasmic loop is supposed to have gained a hydrophobic character, which drove the insertion of this loop into the membrane and resulted in proteins with 14 transmembrane α-helices. Based on this hypothesis, transport proteins with only 6 transmembrane α-helices are expected to be functional as a dimer as the monomer itself is incomplete and inactive, which has been demonstrated for members of the MC family (107,126,131). The smallest functional unit is proposed for the drug/proton antiporter EmrE of *E. coli*. Although negative dominance experiments and binding stoichiometry were not conclusive in excluding a trimeric protein (98,171), the medium resolution projection map obtained from 2-D crystals revealed a dimer (153). All together, this would result in a functional unit of only 8 transmembrane α-helices.

The largest group of secondary transporters is the Major Facilitator Superfamily (MFS), and its members transport a diversity of solutes, ranging from sugars, anions, neurotransmitters to drugs. Also the members of the galactoside-pentoside-hexuronide (GPH) family belong to the MFS. Although the GPH proteins show only marginal sequence similarity to the other members of the MFS, the use of refined search programs clearly suggested that these proteins arose from a common ancestor (107,130). One of the refined search methods comprises hydropathy profile analysis, because it seems that, in general, hydropathy is better conserved within the families of integral membrane proteins than the primary sequence (89).

### *The GPH family.*

The members of the GPH family were previously restricted to bacteria (112), but the proteins also appear to be present in eukarya. Recently, the distantly-related sucrose:$H^+$ symporters of the plant *Arabidopsis thaliana* and a homologue in *Schizosacharomyces pombe*
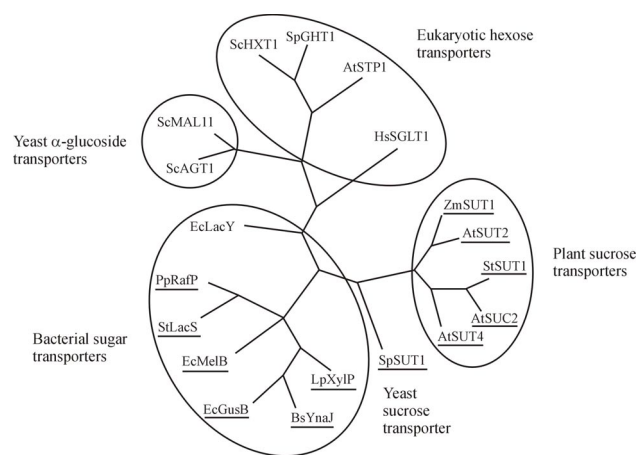
**Fig. 2. Pylogenetic analysis of sugar transporters from bacteria and eukarya.** The picture is taken from Reinders *et al.*, (120). Members of the GPH family are underlined. Bootstrap analysis was performed on aligned amino acid sequences of the *Escherichia coli* lactose transporter (EcLacY), melibiose transporter (EcMelB), and gucuronide transporter (EcGusB); *Pediococcus pentosaceus* raffinose transporter (PpRafP); *Streptococcus thermophilus* lactose transporter (StLacS); *Bacillus subtilus* putative xyloside transporter (BsYnaJ); *Lactobacillus pentosus* xyloside transporter (LpXylP); *Schizosaccharomyces pombe* maltose transporter (SpSUT1) and glucose transporter (SpGHT1); *Arabidopsis thaliana* sucrose transporters (AtSUT4, AtSUC2 and AtSUT2), and monosaccharide transporter (AtSTP1); *Saccharomyces cerevisiae* hexose transporter (ScHXT1), maltose transporter (ScMAL11), and α-glucoside transporter (ScAGT1); *Solanum tuberosum* sucrose transporter (StSUT1); *Zea mays* sucrose transporter (ZmSUT1); *Homo sapiens* glucose transporter (HsSGLT1).

were classified within the GPH-family (120) (Fig. 2). Exciting is the observation of a putative sugar:cation symporter in the eukaryotic *Caenorhabditis elegans*, but for the existence of this protein there is yet no biochemical evidence. Members of the GPH family are all predicted to have 12 transmembrane α-helices. They all transport a unique set of sugars, mostly oligosaccharides, in co-transport with a cation (112) via a transport reaction that can be described as 'alternating access'. This implies that the binding site is only accessible from one side of the membrane at any given time. A full translocation cycle comprises the binding of the cation and sugar at the outside, reorientation of the loaded carrier to the inside, the release of substrate and cation, and the isomerization of

the unloaded carrier back to the initial conformation. Some general features of the bacterial members of the GPH family have been identified by sequence comparisons and mutant analyses. First, the putative transmembrane segments II, IV and XI have a strong periodicity in terms of hydrophilic and hydrophobic residues. The amphipatic character of these transmembrane segments suggests interaction with both hydrophilic and hydrophobic regions, which could represent parts of the translocation pathway and protein-lipid interface, respectively (112). The prokaryotic members of the GPH family have a number of highly conserved negatively and positively charged residues in predicted transmembrane segments. In the putative transmembrane helices II and IV, these residues are aligned with the hydrophilic face of the helices and thereby excluded from the energetically unfavorable membrane environment. For the melibiose carrier, MelB, of *Escherichia coli* and the lactose carrier, LacS, of *Streptococcus thermophilus*, these residues are thought to be involved in cation binding and cation coupling to sugar transport. Second, high sequence conservation is observed in interhelix 10-11. In *S. thermophilus* this loop in suggested to be located in the core of the protein, as electron spin resonance and fluorescence measurements demonstrated that this region is much more rigid than expected for a large cytoplasmic loop (146). Interestingly, a conserved glutamate in this loop (position 379 in LacS) was found to be essential for the coupled transport of proton and sugar (111).

On the basis of analysis of site-directed and second-site suppressor mutants of LacS and MelB, it has been shown that the putative helices I, II, IV, VII, X and XI, and the interhelix loops 6-7 and 10-11 are involved in proton and sugar transport. On the basis of these data, a helix packing model has been proposed by Veenhoff *et al.*, (Fig. 3). The other helices, III, V, VI, VIII, IX and XII, most likely lie outside the core of the protein, as mutations in residues in these transmembrane segments have never been isolated in screens for altered sugar specificity or coupling (160). The suggestion

that interhelix loop 10-11 is located in the core of the protein and is involved in translocation comes from second-site suppressor analysis and solid-state nuclear magnetic resonance (NMR) measurements. The NMR studies indicate that the lysine at position 373 in loop 10-11 is within 15 Å of the anomeric carbon of bound galactose.

A structural feature unique for the transporters of the LacS subfamily concerns the presence of a 20 kDa cytoplasmic domain that is homologous to IIA of various phosphoenol-pyruvate:sugar phosphotransferase systems (PTS). The LacS IIA domain can be phosphorylated by phosphoenolpyruvate (PEP) in the presence of the general PTS proteins, enzyme I and HPr. The IIA domain is not required for lactose transport by LacS but can regulate transport via its phosphorylation state (46). In analogy to LacS, regulation of transport activity of the members of the MelB subfamily involves the cooperative interaction of the soluble IIA protein of the glucose-PTS (IIA$^{Glc}$), which is controlled via the phosphorylation state of IIA$^{Glc}$. Until now, nothing is known about the role of the PTS in the regulation of the members of the GusB subfamily to which the xyloside transporter XylP of *Lactobacillus pentosus* belongs.

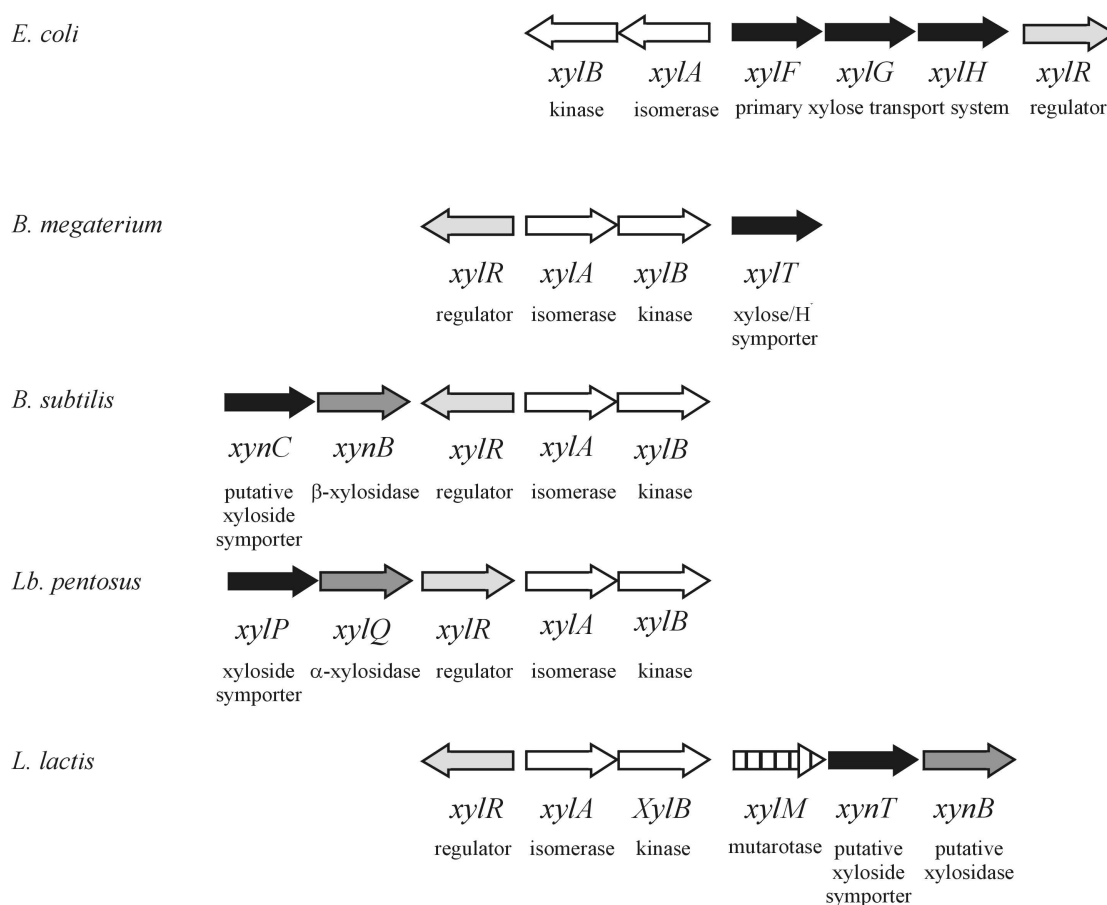## The xyloside transporter XylP of *Lactobacillus pentosus*, a member of the GPH family

### *Xylose metabolism in Lactobacillus pentosus.*

*Lactobacillus pentosus* is a facultative heterofermentative bacterium that is used in the fermentation of vegetables like olives, cucumbers and cabbages (173). Unlike most other *Lactobacillus* species, *Lactobacillus pentosus* is able to use xylose as a sole energy source. Upon transport of xylose, the first steps in the xylose metabolism are identical for all organisms, involving two intracellular enzymes, that is, a D-xylose isomerase (XylA), which isomerizes D-xylose into D-xylulose, and a D-xylose kinase (XylB), which phosphorylates
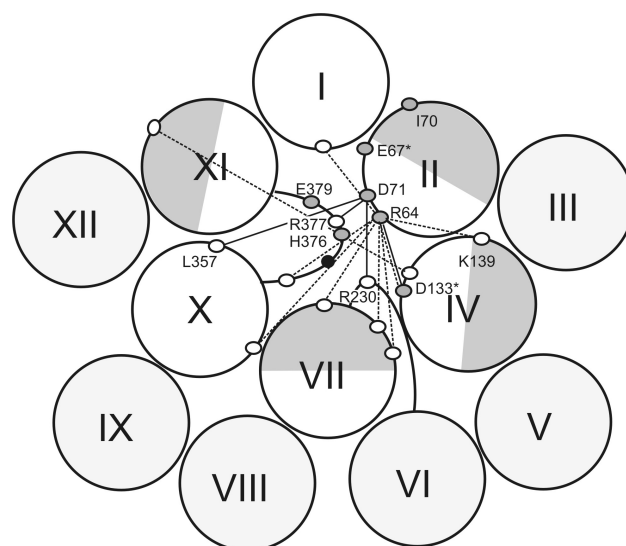


**Fig. 3. Helix packing model of the lactose transport protein, LacS, of *Streptococcus thermophilus.*** The picture is taken from Veenhoff *et al.*, (160) and updated with new data by L. M. Veenhoff. *Continuous lines* connect residues that were identified by second-site suppressor analysis in LacS. *Dashed lines* connect residues that were identified by second-site suppressor analysis in MelB. *The black circle* in loop 10-11 indicates the position of residue 373, which is located within 15 Å from bound galactose. *Stars* indicate residues where substrate binding and/or translocation can protect for site-directed cysteine modification in LacS. Residues that are involved in coupling sugar and proton translocation in LacS are indicated in gray. The hydrophobic faces of amphipathic helices II, IV and XI and the face of helix VII that harbors residues important for the specificity of proton and sugar binding in MelB are *shaded*.

xylulose into xylulose-5-phosphate. In a variety of bacteria, including *Escherichia coli* (144), *Bacillus megaterium* (137), *Bacillus subtilis* (48)*, some *Lactococcus lactis* strains (30), and *Lactobacillus pentosus* (85,86), the genes encoding the D-xylose isomerase and D-xylose kinase, *xylA* and *xylB* genes, are organized in an operon (Fig. 4). The expression of the *xylA* and *xylB* genes is induced in the presence of xylose and is under control of a regulator protein encoded by *xylR*. The mode of regulation can be different for the various organisms. In Gram-negative enteric bacteria, the transcription factor appears to function as a positive regulator (43,81,139,144), whereas in *Lactobacillus pentosus* and other Gram-positive organisms

E. coli

*xylB*    *xylA*    *xylF*   *xylG*   *xylH*   *xylR*

kinase    isomerase    primary xylose transport system    regulator

B. megaterium

*xylR*    *xylA*    *xylB*    *xylT*

regulator   isomerase   kinase    xylose/H⁻ symporter

B. subtilis

*xynC*    *xynB*    *xylR*    *xylA*    *xylB*

putative xyloside symporter   β-xylosidase   regulator   isomerase   kinase

Lb. pentosus

*xylP*    *xylQ*    *xylR*    *xylA*    *xylB*

xyloside symporter   α-xylosidase   regulator   isomerase   kinase

L. lactis

*xylR*    *xylA*    *XylB*    *xylM*   *xynT*   *xynB*

regulator   isomerase   kinase    mutarotase   putative xyloside symporter   putative xylosidase

**Fig. 4. Schematic representation of the organization of the *xyl* operons of various bacteria.** Data are from the following sources: *Escherichia coli* (144), *Bacillus megaterium* (137), *Bacillus subtilis* (48), *Lactobacillus pentosus* (85,86), *Lactococcus lactis* (30).

expression of the *xylAB* operon is negatively regulated by a repressor protein (75,84,127,135,141).

In some organisms, for example *E. coli* (144) and *B. megaterium* (137), the *xyl* operon structure also comprises the gene(s) encoding the xylose transporter (Fig. 4). Until recently, only two mechanisms of xylose transport were known in bacteria, that is, secondary transport in which xylose is co-transported with a proton or a sodium ion (13,20,21,76,137,139,147, 150,154) and high affinity primary transport in which solute accumulation is driven by the hydrolysis of ATP (83,147,149,154). As the *xyl* operon of *Lactobacillus pentosus* also contained a gene encoding a putative transporter, XylP, this protein was initially thought to be involved in xylose transport. However, the observation that another gene of the operon, *xylQ*, encoded an α-xylosidase indicated that XylP could be involved in xyloside transport (14,15).

### Xyloside metabolism in *Lactobacillus pentosus*.

In general, *lactobacilli* do not degrade large polysaccharides such as cellulose and hemicellulose, but readily ferment smaller carbohydrates. As *lactobacillus* species do not have the extracellular enzymes necessary for the degradation of polysaccharides, they are dependent for their substrate supply on the presence of cellulolytic microorganisms. The endoglucanases produced by these organisms act on cellulose and hemicellulose, yielding smaller oligosaccharides like xylosides (167).

The xyloside transporter, XylP, of *Lactobacillus pentosus* is a secondary transport system belonging to the GusB subfamily of GPH transporters (112). The protein is composed of 12 putative transmembrane α-helices with the N- and C-termini located at the cytoplasmic side of the membrane (Fig. 5). The
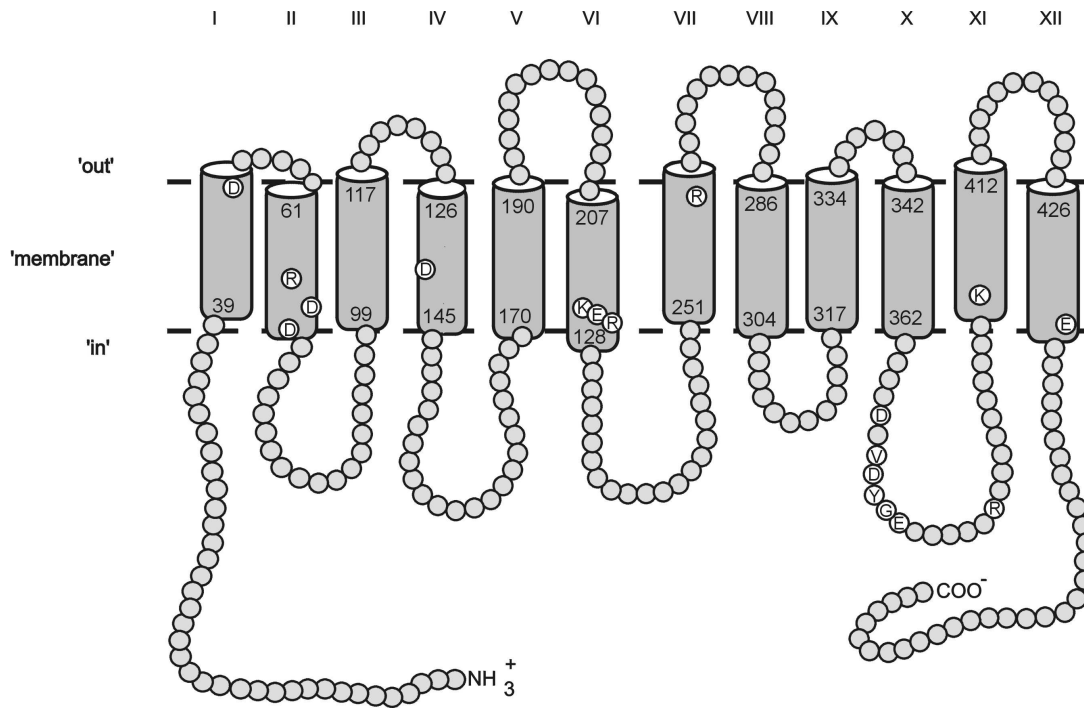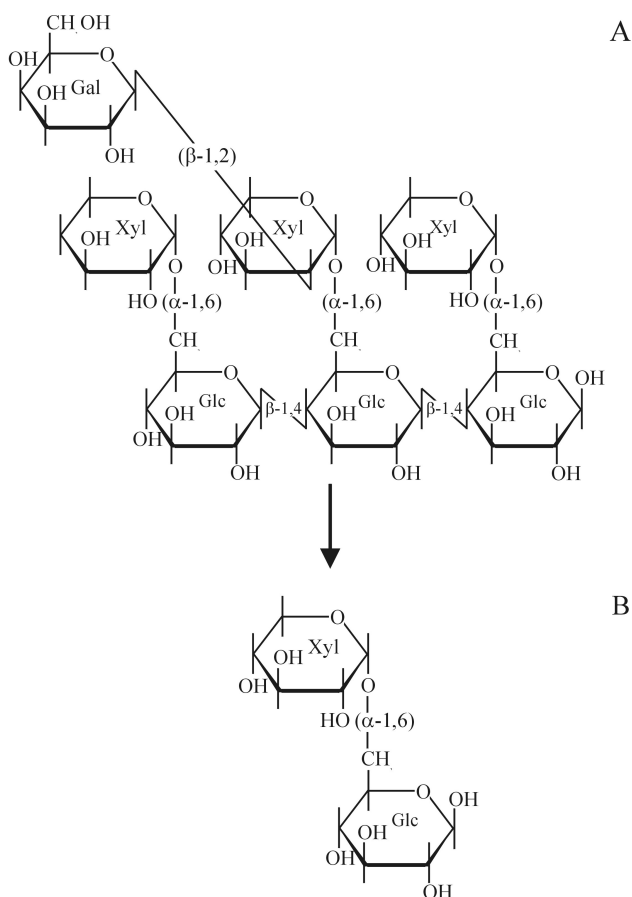
**Fig. 5. Secondary structure model of XylP**. Indicated are the twelve putative transmembrane helices, the charged residues located in the transmembrane segments, and the highly conserved residues in the interhelix loop 10 -11.

XylP protein effects the transport of the xyloside isoprimeverose, a building block of xyloglucan (14,15) (chapter 3), which is the principal hemicellulose component in the primary cell wall of plants and one of the most abundant storage polysaccharides in seeds (Fig. 6). Xyloglucan forms a complex polysaccharide network with cellulose and pectin via a strong hydrogen bonding network. Although abundant in nature, the fate of xylosides in bacterial metabolism is poorly documented. Following transport of isoprimeverose into the bacterial cell, the disaccharide is hydrolyzed by an α-xylosidase, XylQ, (14) into the mono-saccharides xylose and glucose, which are subsequently metabolized via the pentose phosphate pathway. The genes encoding the XylP and XylQ proteins are clustered with those of the xylose metabolism genes (Fig. 4) and are induced in the presence of xylose. Within the GPH family, XylP is most closely related to a putative β-(1,4)-xyloside transporter XynC (YnaJ) of *Bacillus subtilis*, which is like XylP clustered with a gene encoding a xylan β-1,4-

xylosidase and induced by xylose. In many microorganisms homologues of the *xylP* and *xylQ* genes are clustered together and the corresponding proteins are likely to have a role in the uptake and metabolism of xylosides, as has been proposed for the putative xyloside transporter (XynT) and β-1,4-xylosidase (XynB) of *Lactococcus lactis* (30) (Fig. 4).

***Xylose transport in Lactobacillus pentosus.***

As the *xylP* gene does not encode the xylose transporter and the organism is capable of using xylose as a sole carbon source, there must be another protein responsible for the uptake of this sugar. Recently, suggestions have been made that xylose is taken up by facilitated diffusion via the phosphoenolpyruvate: mannose phosphotransferase system (mannose PTS) (16). Evidence for this notion came from spontaneous 2-deoxy-D-glucose-resistant mu-tants, defective in the transport of mannose, which were also impaired in their growth on xylose. In some of the mutants the inability to

**Fig. 6. Structures of (A) xyloglucan and (B) isoprimeverose.**

galactose and fructose via the glucose-PTS of *E. coli* (73,74).

As facilitated diffusion systems do not accumulate substrates in the cell interior and the rate of transport is determined by the subsequent metabolism, this type of transport is not easily characterized with the conventional transport assays that make use of the distribution of radio-labeled substrates. Chapter 2 describes the development of a new spectroscopic transport assay that bypasses many problems associated with radio-labeled distribution methods. With this spectroscopic assay evidence was obtained for facilitated diffusion of xylose in *Lactobacillus pentosus* as will be discussed in chapter 6.

## Structural organization of secondary transport proteins

### *Quaternary structure.*

Membranes are an important site for regulation as they allow the entry and export of solutes and the transduction of signals, and are therefore a suitable place for regulation of these processes at the level of protein-protein interactions. For cytoplasmic proteins, oligomerization is a generally accepted property that is used to regulate enzyme activity or to extend the possible functions of the proteins. A well known example of the importance of oligomerization in protein function is represented by hemoglobin, the oxygen carrier of erythrocytes. Hemoglobin is a tetrameric protein to which oxygen binds in a cooperative manner, meaning that binding of oxygen to one monomer enhances the binding to the others. In addition, metabolites such as 2,3-bisphosphoglycerate but also the pH and the $CO_2$ pressure modulate the cooperative behavior of hemoglobin, which allows the protein to bind $O_2$ with high affinity in the lungs and to efficiently release $O_2$ in actively metabolizing tissues (148).

Innumerous opportunities for regulation are possible through the interaction of cytoplasmic proteins with membrane proteins.

grow on xylose could be complemented by the IIB protein of the mannose-PTS of *Lactobacillus curvatus*, supporting the idea that the mannose-PTS is involved in xylose transport. As in other mutants growth on xylose could be restored by complementation with the xylose transporter of *Lactobacillus brevis*, defects in genes involved in xylose metabolism could be excluded. PTS systems catalyze the concomitant transport and phosphorylation of sugars at the expense of phosphoenol pyruvate (PEP). The inability to detect PEP-dependent phosphorylation of xylose suggests that the sugar enters the cell by facilitated diffusion. In literature, other examples of PTS systems involved in facilitated diffusion of sugars are known, like the transport of D-galactose and trehalose via the mannose-PTS of *S. typhimurium* (116,117), and the transport of

# Chapter 1

An example is for instance the IIA protein ($IIA^{Glc}$) of the glucose-PTS in enteric bacteria. $IIA^{Glc}$ not only interacts with the components of the PTS in order to catalyze concomitant glucose transport and phosphorylation, but the protein also plays a key role in the regulation of non-PTS carbohydrate transporters and metabolic enzymes. In Gram-negative enteric bacteria this regulation involves the allosteric interaction of unphosphorylated $IIA^{Glc}$ with non-PTS transporters, such as the ion-linked transporter for melibiose (MelB), lactose (LacY) and raffinose (RafP), the ATP-binding-cassette transporter for maltose (MalK), and glycerol kinase of *E. coli* (60,125,129,155,159, 163). In the phosphorylated state $IIA^{Glc}$ is involved in transcriptional control by stimulating the activity of adenylate cyclase. Unfortunately, there is no solid biochemical evidence for the interaction between $IIA^{Glc}$ and adenylate cyclase (23,119).

Recently, it has been shown that the membrane-bound IICB complex of the glucose-PTS of *E. coli* is involved in transcriptional control, as glucose induction is mediated by the direct physical interaction between the $IICB^{Glc}$ complex and the Mlc (making large colonies) protein, depending on the phosphorylation state of the $IICB^{Glc}$ complex. Free Mlc can bind to and repress its target genes such as those for the glucose PTS proteins. In the presence of glucose, dephosphorylated $IICB^{Glc}$ recruits Mlc and sequesters it from binding to its target promoters, resulting in a derepression of gene expression (100).

In recent years, it has become apparent that membrane proteins do not operate as free-floating entities, but rather they cooperate in larger oligomeric complexes. The best known examples are from channel proteins. The eukaryotic water-conducting channels (aqua-porins) and the *E. coli* glycerol facilitator, which are all members of the Major Intrinsic Protein Family, assemble in the membrane as tetramers of four independent channels. The suggestion has been made that the central pore between the four subunits can form an ion-channel (29,37,143). A similar arrangement has been proposed for the human neural glutamate transporter, EAAT3, which functions both as a glutamate transporter and a glutamate-gated chloride channel (31). For the glycerol facilitator it has also been speculated that the protein exists in close association with glycerol kinase, which would tune the uptake and ATP-dependent phosphorylation of glycerol (92,163). Also the eukaryotic ligand-gated ion channels are oligomeric complexes that interact with specific cytoplasmic proteins, through which linkage to the cytoskeleton and to intracellular signal transduction pathways is possible (140). Another example concerns the $K_{ATP}$ channels, as they are a combination of transport ATPases complexed with potassium channel subunits. $K_{ATP}$ channels are composed of four sulfonylurea receptors (SURs), members of the ATP-binding cassette (ABC) transporter family, and four potassium inward rectifiers (Kir6.1 and Kir6.2), that assemble to form a large octameric complex. Opening of the channels has been proposed to be regulated via the ABC transporter subunits (10).

The overall impression is that both homo- and hetero-oligomer formation of proteins in the membrane plays an important role in nature. At the functional level, it can create possibilities for scaffolding and allostery. Structurally, there might be a reason as well for multimerization, as the restrictive volume of the 2-dimensional space enhances self-association, allowing a more efficient packing in the membrane (44).

In view of the notion that the membrane is a site particularly well suited for regulation at the level of protein-protein interactions, it is unlikely to assume that secondary membrane transport proteins would solely operate as monomeric units. The quaternary structure and function of oligomerization of secondary transport proteins will be evaluated in chapter 5 of this thesis.

*Quaternary structure analysis of proteins of the GPH family.*

So far, the lactose transporter LacS of *S. thermophilus* is the only member of the GPH family for which the quaternary structure has been elucidated. LacS catalyzes the uptake of a variety of galactosides either in symport with $H^+$, proton motive force-driven uptake, or in exchange for another sugar. Analytical ultracentrifugation, freeze-fracture electron microscopy (36), rotational correlation spectroscopy (145), and negative-dominance analysis (161) revealed that this protein is organized as a dimer, in which each subunit harbors a full translocation pathway. Co-operativity, indicative of functional subunit interaction, was observed in the proton motive force-driven uptake reaction but not in exchange transport. On the basis of these results, it has been proposed that coupling of the two LacS monomers is associated with the reorientation of the empty carrier, a step unique for proton motive force-driven uptake. Characterization of a member of the GPH family without the soluble regulatory domain present in LacS, could give more insight in the structural organization of this family of proteins and the domain(s) involved in dimerization. The quaternary structure analysis of the xyloside transporter XylP of *Lactobacillus pentosus* is presented in chapter 4.

*Hetero-oligomer formation within the GPH-family.*

Does hetero-oligomerization play a role in the regulation of transport reactions catalyzed by proteins of the GPH family? Although XylQ is a cytosolic protein, α-xylosidase activity is associated with the membrane fraction of the cell. This could either mean that XylQ has a high affinity for the membrane lipids or that the protein specifically interacts with the transporter XylP (14). A direct interaction between a transporter and the first metabolic enzyme has been observed for LacS and β-galactosidase (LacZ) of *S. thermophilus* as will be discussed in chapter 6. *In vivo*, LacS catalyzes the internalization of lactose, which, subsequently is hydrolyzed by β-galactosidase, yielding the monosaccharides glucose and galactose. Glucose is metabolized but the majority of *S. thermophilus* strains cannot utilize the galactose moiety, even though the genes necessary for galactose metabolism are present. In stead, galactose is used as a counter-substrate in the exchange reaction with lactose, and this mode of transport occurs both in Gal⁻ and Gal⁺ cells. The overall reaction is a lactose/galactose exchange without the net movement of protons. This mode of transport is about an order of a magnitude faster than the lactose-$H^+$ symport reaction. Since the galactose unit of lactose is the part that is recognized by the LacS protein (162) and this monosaccharide is subsequently used in the second half of the exchange reaction, it would be most favorable when hydrolysis of the lactose would occur near the cytoplasmic sugar binding site of LacS. In order to achieve this, the LacS and LacZ could form a hetero-oligomeric complex. With the use of Blue Native Polyacrylamide Gel Electro-phoresis (BN-PAGE), a technique that is described in chapter 4 of this thesis, we were able to show that LacS and β-galactosidase are associated in a complex when *S. thermophilus* membrane vesicles were solubilized with detergent (Heuberger, Veenhoff and Duurkens, unpublished results). Despite the fact that the data are still preliminary and a functional role of the LacS-LacZ interaction has not yet been established, it could represent a nice example of how hetero-oligomer formation increases metabolic efficiency.

## Outline of this thesis

Transport processes are essential for the existence of all organisms as transport proteins represent the gateway for solutes into or out of the cell. As a consequence, transporters are also the cause of many problems when they are hampered in their functioning (diseases,

biotechnological conversions etc.). Insight in the mechanistic features and structural organization of these proteins is therefore of broad interest and importance. This thesis makes a contribution to the understanding of the mechanisms of secondary transport proteins through a description of the functional and structural properties of the xyloside transporter XylP of *Lactobacillus pentosus*. In addition, the work describes some new and improved methodologies for the characterization of transport reactions and quaternary structure analysis of membrane proteins.

As the natural substrate of XylP, the disaccharide isoprimeverose, was not commercially available, nor easily isolated from cell wall material, the carbohydrate was chemically synthesized as described in chapter 3. Chapter 2 describes the development of a new spectroscopy-based transport assay for the analysis of carbohydrate transport reactions. An assay that is especially useful for those carbohydrate transporters for which radio-labeled substrates are not available, for the analysis of uniport transport reactions and/or for the characterization of mutants in which the transport of sugar and cation (proton) are uncoupled. In chapter 3, this spectroscopic assay was applied to study the transport of isoprimeverose by the XylP protein, which first had to be purified and reconstituted into an artificial membrane system.

As long as it is extremely hard, so far impossible, to obtain high resolution structural data of secondary transporters, one has to rely on methods that provide a low or medium resolution structure, specific information on ligand binding-site, subunit interactions, etc. In chapter 4, the use of Blue Native Page Gel Electrophoresis for the analysis of quaternary structure is evaluated, and the technique was used in combination with analytical ultracentrifugation and freeze-fracture electron microscopy to determine the oligomeric structure of XylP. Chapter 5 critically evaluates the techniques that are available to study the quaternary structure of membrane proteins, and it provides an overview of the quaternary structures and the oligomeric function of secondary transporters studied so far.