J. Membrane Biol. 138, 1–11 (1994)

The Journal of
Membrane
Biology

Topical Review

Function and Presumed Molecular Structure of Na^+-D-Glucose Cotransport Systems

H. Koepsell, J. Spangenberg
Anatomisches Institut der Universität Würzburg, Koellikerstr.6, 97070 Würzburg, Germany

Received: 10 June 1993/Revised: 23 September 1993

Abstract. Functional characterization of Na^+-D-glucose cotransport in intestine and kidney indicates the existence of heterogeneous Na^+-D-glucose cotransport systems. Target size analysis of the transporting unit and model analysis of substrate binding have been performed and proteins have been cloned which mediate (SGLT1) and modulate (RS1) the expression of Na^+-D-glucose cotransport. The experiments support the hypothesis that functional Na^+-D-glucose cotransport systems in mammals are composed of two SGLT1-type subunits and may contain one or two RS1-type proteins. SGLT1 contains up to twelve membrane-spanning α-helices, whereas RS1 is a hydrophilic extracellular protein which is anchored in the brush-border membrane by a hydrophobic α-helix at the C-terminus. SGLT1 alone is able to translocate glucose together with sodium; however, RS1 increases the V_{max} of transport expressed by SGLT1. In addition, the biphasic glucose dependence of transport, which is typical for kidney and has been often observed in intestine, was only obtained after coexpression of SGLT1 and RS1.

Introduction

In the late 1950's, sodium-dependent glucose uptake was detected in intestinal epithelial cells [17, 83], and in 1960, Crane [15] formulated the hypothesis that the uptake may be energized by the Na^+-gradient across the cell membrane. Since that time, Na^+-D-glucose cotransport has been extensively investigated by transport and binding measurements and by biochemical procedures (for earlier reviews, *see* [12, 47, 53, 54, 87, 91, 93, 109]). By cloning an integral membrane protein from rabbit small intestine (SGLT1) which mediates Na^+-coupled transport of D-glucose [34], Wright and collaborators made an important step in investigating Na^+-D-glucose cotransport on a molecular basis. SGLT1 was also detected in kidney [68] and SGLT1-homologous clones, which mediate Na^+-dependent transport of nucleosides [77], *myo*-inositol [56] and alanine [55], were isolated. Functional studies on SGLT1-protein expressed in different systems [6, 7, 42, 94] showed that SGLT1 expresses Na^+-D-glucose cotransport which exhibits a Michaelis Menten-type transport kinetic and one type of phlorizin inhibition site. The characteristics of the expressed transport are different from those observed in vivo since high and low affinity transport and binding sites of glucose and phlorizin were detected in different regions of kidney and in intestine [19, 25, 31, 45, 51, 60, 61]. Using a monoclonal antibody which alters phlorizin binding to the Na^+-D-glucose cotransport system, we recently cloned a membrane-associated protein which changes the Michaelis Menten-type glucose dependence of Na^+-D-glucose cotransport expressed by SGLT1 to the complex glucose dependence observed in vivo and alters the voltage dependence of glucose transport [96, 111, 112].

In this review, we compile functional data which characterize the properties of Na^+-D-glucose cotransport systems in kidney and intestine. In addition, genetic and biochemical experiments are summarized in which components of Na^+-D-glucose cotransport systems were

Key words: Glucose transport — Sodium-dependent cotransport — Molecular structure — Subunit composition — Transporter heterogeneity

Correspondence to: H. Koepsell

identified and described. Since the subunit composition of the Na^+-D-glucose cotransporter has not been completely determined and the molecular basis for the heterogeneity of Na^+-D-glucose cotransporters in different localizations has not been defined, the available data serve as basis to develop a hypothesis on the molecular structure of Na^+-D-glucose cotransport systems and to speculate on structural elements which are responsible for transporter heterogeneity.

The Basic Functional Properties of Na^+-D-Glucose Cotransport Systems Have Been Determined

For more than thirty years sodium-dependent glucose transport has been studied. Early uptake measurements performed with tissue slices, with whole organs or by micropuncture techniques elucidated the sodium dependence, substrate specificity, electrogenicity and phlorizin inhibition of sodium-dependent glucose uptake (for reviews, *see* [53, 89, 91]). After methods were developed to measure transport in membrane vesicles with controlled solutes on both sides of the membrane, it was demonstrated that sodium gradients energize the uptake of D-glucose and that glucose gradients may drive sodium uptake [39, 50, 70]. The glucose and the sodium dependence of the transport was characterized and the stoichiometry between cotransported sodium and D-glucose was determined, using brush-border membrane vesicles from renal proximal tubules and small intestine [46, 49, 106–108]. The transporter translocates D-glucose together with one or two sodium ions, and is not influenced by anions. It accepts sugar molecules in the $^4C_1$ chair conformation of D-glucose and the OH groups at C_2, C_3, C_4, and C_6 appear to be important for transport [90, 92, 110]. Phlorizin, a polycyclic glycoside, is a competitive inhibitor of the Na^+-D-glucose cotransporter which binds only to the extracellular side of the transporter and is not translocated (for review, *see* [53]). Competitive inhibition of phlorizin binding by D-glucose and by the phloretin moiety of phlorizin suggests that phlorizin interacts not only at the substrate binding site but also at a nearby hydrophobic protein domain [51]. The dissociation constant of phlorizin binding is more than two orders of magnitude smaller than the apparent $K_{0.5}$ for D-glucose uptake. It is decreased by sodium and (in the presence of sodium) by an inside negative membrane potential which may increase the affinity of sodium binding to the transporter [82]. The inside negative membrane potential accelerates the onset of phlorizin binding but does not alter the debinding of phlorizin [2, 103]. Recently, different models were fitted to families of onset-curves of phlorizin binding to brush-border membrane vesicles from porcine renal outer cortex (early segments of proximal tubules) and outer medulla (late segments of proximal tubules) [51]. For a good fit, two coexisting Na^+-dependent phlorizin binding sites with a stoichiometry of 1:1 had to be assumed in both kidney regions.

Genetic Diseases and Functional Data Suggest the Existence of Heterogeneous Na^+-D-Glucose Cotransporter Systems

Mammals are supposed to contain two or more heterogeneous Na^+-D-glucose cotransport systems since in man genetic defects have been observed in which renal and intestinal transport is preferentially or selectively impaired. In glucose-galactose malabsorption, small intestinal transport is out of action, whereas renal transport is only slightly affected [20, 59, 64]. At variance in renal glycosuria, Na^+-D-glucose cotransport in the kidney is defective and the small intestinal transport is not altered [20, 86]. The data suggest that two or more structurally different Na^+-D-glucose cotransporters are present in kidney and that one of these is identical to the small intestinal transporter. Two Na^+-D-glucose cotransport systems with some difference in substrate specificity were also suggested from glucose uptake measurements which were performed by micropuncture along the proximal tubule and by uptake measurements in brush-border membrane vesicles isolated from small intestine or from proximal tubules of different kidney regions [3, 31, 81, 106, 107]. One Na^+-D-glucose cotransport system which has been described as a low affinity high capacity system, was observed in outer renal cortex. It exhibits a high apparent half-maximal activation by D-glucose, a relatively high maximal velocity of uptake, and shows an apparent 1:1 stoichiometry of sodium and glucose. The other system which has been described as a high affinity low capacity system, exhibits a lower apparent half-maximal activation by D-glucose and a smaller V_{max} value. This system exists in small intestine and in outer renal medulla (late segments of proximal tubules) and has an apparent Na^+/glucose stoichiometry of 2:1.

Recent glucose transport and phlorizin binding measurements showed that the situation is more complex. Thus, transport measurements in brush-border membrane vesicles from pig and rat kidney showed that high and low affinity uptake can be distinguished in both outer cortex and outer medulla. Therefore, appropriate experimental conditions have to be used and a sufficiently large range of substrate concentrations has to be tested [8, 51]. The low affinity transport activity predominates in outer cortex and the high affinity transport activity in outer medulla. Since phlorizin binding measurements showed that two types of sodium-dependent high affinity phlorizin binding sites exist in both kidney regions with a stoichiometry of about 1:1, the hypothesis was raised that each functional transport molecule

contains a low and high affinity substrate site [51]. Small structural differences of transporter components in different kidney regions and different small intestinal segments may be responsible for the observed heterogeneity in transport.

There is an ongoing discussion whether heterogeneous Na^+-D-glucose cotransport systems exist in small intestine. Thus, some authors observed low and high affinity transport in fetal but not in adult small intestine [60, 61] whereas others described low and high affinity transport also in the adult [10, 11, 19, 25, 31, 42]. Notwithstanding that there may be species differences and that in some studies an inappropriate data analysis may have led to an overestimation of transport heterogeneity [62], the combined experimental evidence suggests that heterogeneous transport activity also exists in intestine. Heterogeneous transport may be only detectable under some optimal experimental conditions which may be species dependent. Thus, at variance to porcine outer medulla, we did not detect heterogeneous Na^+-D-glucose transport in porcine outer cortex when the membrane potential was clamped to zero (*see* Fig. 11 in [51]). As shown in kidney and also in intestine, heterogeneous transport activity is supposed to be due to coexisting low and high affinity substrate binding sites since also in intestine two types of Na^+-dependent phlorizin binding sites have been observed [23] which appear to be present in the tips of the microvilli and in the crypts (*see* biphasic Scatchard plots in Fig. 3 of [24]).

Transporter Components with Molecular Masses around 70 kD Have Been Identified

Many attempts have been made to identify components of Na^+-D-glucose cotransport systems by labeling experiments with side-group specific reagents, covalently binding substrate analogues and monoclonal antibodies (for review, *see* [53]). That a polypeptide with a molecular mass around 70 kD is a component of the intestinal Na^+-D-glucose cotransport system was first suggested from experiments of Semenza and coworkers [27, 41, 85] and was demonstrated more unambiguously by Peerce and Wright [78, 79] who used affinity labeling with the side-group specific reagent fluorescein isothiocyanate. Components of renal Na^+-D-glucose cotransport systems were identified by affinity labeling with a covalently binding D-glucose analogue [73, 74] and by monoclonal antibodies which alter Na^+-D-glucose cotransport and/or high affinity phlorizin binding and react in Western blots [52, 117]. Also in these studies, a polypeptide with an apparent molecular mass around 70 kD was identified as a component of the transporter. A polypeptide with a molecular weight of around 45,000, which was also labeled by the glucose analogue and by several monoclonal antibodies, may be a splitting product of the M_r 70,000 polypeptide. Some experiments suggested heterogeneity of the labeled M_r 70,000 polypeptide [74]. However, since the resolution of hydrophobic and glycosylated proteins in SDS polyacrylamide gels is limited, it cannot be decided whether one, two, or several polypeptides have been identified.

An Integral Membrane Protein (SGLT1) Has Been Cloned which Translocates Sodium Together with D-Glucose

Using the method of expression cloning, Wright and coworkers [34] cloned an integral membrane protein which performs cotransport of sodium and glucose. Starting from poly(A)$^+$ mRNA of rabbit intestine which increased the Na^+-dependent methyl-α-D-glucopyranoside (AMG) transport in oocytes of *Xenopus laevis* over the endogeneous transport activity [113, 114], they isolated an mRNA fraction with an increased transport-expressing activity [35] and prepared a cDNA-library which was screened for clones that mediate Na^+-dependent AMG uptake. A clone termed SGLT1 was isolated which increased AMG uptake in *Xenopus* oocytes drastically. Since by SGLT1 Na^+-D-glucose cotransport was also expressed in systems where no endogeneous transport has been detected [6, 7, 94], SGLT1 protein is most probably sufficient to perform cotransport of sodium and D-glucose. SGLT1 encodes a protein containing eleven or twelve putative membrane-spanning α-helices. It contains 662 amino acids, has a molecular mass of 73,080 D and is glycosylated at asparagine 248 [36]. SGLT1 was originally isolated from rabbit intestine but occurs also in kidney where the expression of an identical protein was detected [14, 68]. From human intestine, Wright and coworkers [37] cloned a SGLT1-homologous protein which contains 84% amino acids identical to SGLT1 and was also capable of expressing highly active Na^+-D-glucose cotransport [37]. This gene was localized on the distal q arm of chromosome 22 [33]. In two children from one family which suffer from the recessive disease glucose/galactose malabsorption, a unique homozygous single base exchange was detected which results in an exchange of aspartate at position 28 to asparagine [104]. Since in glucose/galactose malabsorption the intestinal transport is abolished, whereas renal transport is only slightly impaired, the data show that SGLT1 is an indispensible part of the intestinal Na^+-D-glucose cotransport and that the bulk of renal D-glucose reabsorption is performed by a different system. When the glucose dependence and the phlorizin inhibition of SGLT1-expressed Na^+-D-glucose cotransport was measured, a simple Michaelis-Menten kinetic and one type of phlorizin inhibition site were always obtained [6,

42, 111, 112]. This is apparently different from the situation in vivo where transport kinetics with two D-glucose activation sites and two phlorizin binding sites were observed in different localizations (*see above*).

The Functional Molecular Mass of Na^+-D-Glucose Cotransport Systems Is around 300 kD

To determine the functional molecular mass of the intestinal and renal Na^+-D-glucose cotransport system, radiation inactivation experiments have been performed by many laboratories. For the functional unit of phlorizin binding, a molecular mass of 110,000 or 230,000 has been reported [58, 105] whereas the molecular mass for Na^+-D-glucose cotransport was consistently determined to be around 300,000 [4, 58, 98, 101]. Together with the data obtained from affinity labeling and cloning of Na^+-D-glucose cotransporter components, the results from radiation inactivation suggest that the functional Na^+-D-glucose cotransport systems in intestine and kidney are oligomeric proteins and that not all components of the systems are essential for phlorizin binding. The kinetic data on sodium-dependent glucose transport and phlorizin binding, together with the complex effects of D-glucose and phlorizin on affinity labeling of the renal Na^+-D-glucose cotransport system by a covalently binding analogue of cyclosporin [51, 119], suggest that the transporter contains two substrate binding components which are supposed to be SGLT1-type polypeptides. Since we have cloned a protein with a molecular mass around 70 kD (RS1) which interacts with SGLT1-type proteins and alters Na^+-D-glucose cotransport expressed by SGLT1 (*see below*), the Na^+-D-glucose cotransport system is anticipated to be a hetero-oligomer which contains SGLT1-type and RS1-type polypeptides.

SGLT1-Homologous Proteins Mediate Na^+ Cotransport of Nucleosides, Alanine, and *Myo*-inositol

Some sequence similarity between SGLT1 and the bacterial Na^+-dependent transporters for proline and pantothenate [37, 43, 72] suggests common ancestry. In the last few years, several clones with high homology to SGLT1 were isolated from mammalian tissues which are members of a family of Na^+-cotransport systems (Table). Three other families of Na^+ cotransporters have been described. One family is homologous to the Na^+-gamma aminobutyric acid cotransporter GAT1 and contains Na^+ cotransporters for different neurotransmitters, a Na^+-betaine cotransporter and a Na^+-choline cotransporter [28, 84]. The second family contains three different Na^+-L-glutamate cotransporters and has common ancestry with glutamate transporters of bacteria [44, 80, 100], whereas a Na^+-phosphate cotransporter [116] belongs apparently to a third family. The members of the SGLT1-family (Table) were identified by hybridization with SGLT1 or by expression cloning. For two of the clones (Hu14, SNST1) which were identified by hybridization, some functional activity could be demonstrated. Hu14 is probably derived from kidney cortex and mediates small activity of Na^+-dependent glucose transport in *Xenopus* oocytes [115]. Thus, Hu14 may be a component of the low affinity Na^+-D-glucose cotransport system from renal cortex but it may be also a component of a Na^+-cotransport system with a nonidentified main substrate. Since SNST1 mediates some Na^+-dependent uridine uptake, it is supposed to be a component of a Na^+-nucleoside cotransport system. The SGLT1-homologous clones identified by expression cloning showed high expression of Na^+-dependent uptake of *myo*-inositol [56] and alanine [55]. The data show that the SGLT1 family contains different Na^+ cotransporters and is not restricted to Na^+-cotransport systems for sugars.

Table. Members of the SGLT1-family

Clone (origin)	Ident. AS with SGLT1	Function	Tissue distribution
SGLT1 (rabbit)		Cotransp. of Na^+ and glucose	Small intestine, kidney: outer medulla, cortex
Hu14 (man)	58%	Unknown, cotransp. of Na^+ and glucose	Kidney: cortex, outer medulla small intestine
SNST1 (rabbit)	61%	Cotransp. of Na^+ and nucleosides	Kidney cortex, heart muscle
SMIT (dog)	46%	Cotransp. of Na^+ and *myo*-inositol	Kidney: inner medulla, cortex brain
SAAT1 (pig)	74%	Cotransp. of Na^+ and amino acids	Kidney, small intestine, spleen liver, skeletal muscle

The cDNA-sequences of clones are reported in references 34 (SGLT1), 115 (Hu14), 77 (SNST1), 56 (SMIT) and 55 (SAAT1).

Na^+-Cotransport Systems for Glucose and Amino Acids Are Supposed to Interact

In the 1970's, Alvarado [1] raised the hypothesis of a "polyfunctional carrier for Na^+-coupled transport" on the basis of data which showed inhibition of Na^+-dependent amino acid uptake by sugars. Since it could not be excluded that the observed interactions between sodium-dependent sugar and amino acid uptake were indirectly mediated by mutual effects on the membrane po-

tential and/or the sodium gradient [48, 71], this hypothesis was dropped until immunological experiments showed that amino acids are able to alter the structure of the Na^+-D-glucose cotransporter under equilibrium conditions [52]. These studies were performed with a monoclonal antibody (R5A3 in [52]) which is directed against a component of the Na^+-D-glucose cotransporter since it inhibits Na^+-dependent D-glucose uptake and high affinity phlorizin binding in brush-border membrane vesicles. In the presence of Na^+, binding of R5A3 to brush-border membrane proteins was increased by D-glucose but not by D-mannose. In the absence of D-glucose, antibody binding was not influenced by L-alanine. However, if D-glucose was present L-alanine reduced the binding of R5A3 in a concentration-dependent manner. The data show that R5A3 binds to an epitope of the Na^+-D-glucose cotransporter which undergoes conformational changes induced by L-alanine. We have started experiments to investigate the effect of L-alanine on Na^+-D-glucose cotransport in which indirect effects via the membrane potential and/or Na^+ gradients are avoided. Thus, poly(A^+)mRNA from rat intestine was injected into oocytes of *X. laevis* and the influence of L-alanine on sodium-dependent uptake of methyl α-D-glucopyranoside (AMG) was measured under conditions where the Na^+-gradient was not altered and the membrane potential was clamped. In these experiments, Na^+-D-glucose cotransport was stimulated by L-alanine in a concentration-dependent manner (*unpublished data*). Together with the antibody data, these results strongly suggest functional interactions between the Na^+-cotransport systems for D-glucose and L-alanine.

Na^+-D-Glucose Cotransport Systems Are Regulated

The regulation of Na^+-D-glucose cotransport has been investigated in $LLCPK_1$-cells which are derived from kidney [66, 67, 76] and in small intestine [10, 18, 21, 24, 32, 65, 88]. In both systems, carbohydrates induce opposite effects on Na^+-D-glucose cotransport. Since the regulation of a cell line observed in tissue culture may not represent the complex regulatory process in vivo, we restrict our discussion to the data from small intestine. In small intestine Na^+-D-glucose cotransport is upregulated during streptozotocin-induced diabetes [9, 65], by the hormones 1,25-dihydroxycholecalciferol and 17α-methyltestosterone [18, 32] and by carbohydrate-rich diets [21, 22, 24, 88].

Before discussing the diet-induced regulation, some remarks on the normal distribution of the intestinal Na^+-D-glucose cotransporter should be made. Thus, Na^+-D-glucose cotransport has been detected in duodenum, jejunum and ileum. The highest maximal velocity of transport, concentration of phlorizin binding sites and concentrations of transporter components were observed in mid-jejunum where the D-glucose concentration is normally higher than in the other small intestinal segments [16, 29, 40]. Along the crypt-villus axis where the enterocytes undergo increasing stages of maturation, different functional activities of the Na^+-D-glucose cotransporter and different concentrations of transporter components and related mRNAs were detected. Already in the crypts, some mRNA with homology to the recently cloned Na^+-D-glucose transporting protein SGLT1 was found [26, 95], some antibody binding to transporter components was observed (*see* [29]) and Fig. 2*b* in [102]) and some Na^+-dependent phlorizin binding was measured [21, 24]. At variance, no Na^+-D-glucose cotransport activity could be detected in the crypts [95, 99]. Phlorizin binding and SGLT1-homologous mRNA increased rapidly at the crypt-villus junction [24, 26, 95], whereas Na^+-D-glucose cotransport activity starts at the mid-villus region and increases towards the tips of the villi. The data show that Na^+-D-glucose cotransport is neither strictly correlated to phlorizin binding nor to the concentration of SGLT1-homologous mRNA.

Carbohydrate-rich diets stimulate Na^+-D-glucose cotransport activity, phlorizin binding and the amount of SGLT1-homologous mRNA [21, 24, 88]. The sugar-induced stimulation of transport activity is supposed to be due to an increase of transporter density since some parallel increase of the V_{max} of transport and of phlorizin binding sites was observed [22]. This upregulation is post-transcriptional since it is correlated with the concentration of SGLT1 protein in the plasma membrane [88] but not with the amount of SGLT1-homologous mRNA [57]. The post-transcriptional regulation may be due to chemical modification(s) of SGLT1 which may alter membrane insertion and/or protein turnover. Another possibility is that the regulation is mediated by a second component of the transport system. We favor this explanation since we have cloned a protein which associates with SGLT1-type proteins and induces apparent high and low affinity D-glucose transport sites. It has also been shown that in vivo during starvation, high and low affinity Na^+-dependent glucose transport activities are differentially altered [10]. Also, data have been reported which show that the relationship between high and low affinity phlorizin binding sites in mouse small intestine changes during a carbohydrate-rich diet (*see* Fig. 3*b* in [24]).

Na^+-D-Glucose Cotransport Systems Probably Contain Regulatory Subunits

Recently, monoclonal antibodies against Na^+-D-glucose cotransport systems were prepared which alter

sodium-dependent D-glucose transport and/or sodium-dependent high affinity phlorizin binding [30, 38, 52, 117]. One monoclonal antibody directed against a brush-border membrane protein with an apparent molecular weight around 70,000 (*see* R4A6 in [52]) increased high affinity phlorizin binding to renal and intestinal brush-border membrane proteins but did not bind to expressed SGLT1 protein. To characterize the antigenic protein of R4A6, an expression library of porcine kidney cortex was screened. A cDNA clone (eventually called RS1) was obtained which encodes a protein with 623 amino acids and has a molecular mass of 66,832 [96, 112]. RS1 protein turned out to be relatively hydrophilic and to contain one single hydrophobic α-helix at the very C-terminus which is supposed to be the membrane-anchor of RS1. This α-helix could be membrane spanning. In this case, the two N-terminal amino acids of RS1 are predicted to be exposed to the intracellular side. By expression of RS1 in reticulocyte-lysates, a polypeptide with an apparent molecular mass of 70 kD was obtained which was specifically recognized by R4A6. Since R4A6 binds to the extracellular side of the renal and intestinal brush-border membrane [29, 52], the predominant part of RS1 is localized on the extracellular membrane side. Immunohistochemistry with antibodies against RS1 and SGLT1 showed that RS1 (or RS1-homologous proteins) and SGLT1 (or SGLT1-homologous proteins) are localized in the brush-border membrane [112]. Native polyacrylamide gels revealed that antibodies against RS1 and SGLT1 react with a polypeptide oligomer with a molecular mass around 300 kD. This suggests that SGLT1-type and RS1-type proteins form oligomers in the membrane.

RS1 was expressed in oocytes of *X. laevis*. Non-injected oocytes exhibit low activity of endogeneous Na^+-D-glucose cotransport [113, 114] which varies in different batches of oocytes. The endogeneous glucose transport is supposed to be mediated by a transport system containing SGLT1-type transporting proteins but no RS1-type subunit(s), since oocytes contain SGLT1-homologous mRNA but no mRNA with homology to RS1 [112]. After injecting RS1-cRNA into *Xenopus* oocytes, the low endogeneous Na^+-D-glucose cotransport was increased. Immunohistochemistry with antibody R4A6 showed that expressed RS1 protein is incorporated into the plasma membrane. To investigate more specifically whether expressed SGLT1 interacts with expressed RS1, coinjection experiments with RS1-cRNA and SGLT1-cRNA were performed, and the effect of RS1 on glucose transport expressed by SGLT1 was determined. When RS1-cRNA was injected with a small amount of SGLT1-cRNA, an up to 40-fold increase of expressed Na^+-D-glucose cotransport was observed. Stimulation of expressed Na^+-D-glucose cotransport was observed when the molar ratio between injected RS1-cRNA and SGLT1-cRNA was around 2, whereas no effects were detected when the molar ratio between the injected cRNA's of RS1 and SGLT1 was below 0.5 or above 8 [112]. We were able to demonstrate that the effects of RS1-cRNA on glucose transport are due to an interaction of RS1 and SGLT1 protein in the oocyte plasma membrane by showing that the transport kinetics and potential dependence of Na^+-D-glucose cotransport expressed by SGLT1 was altered by coinjection of RS1-cRNA. Thus, measuring the AMG dependence of expressed Na^+-D-glucose cotransport after injection of different amounts of SGLT1-cRNA, a simple Michaelis-Menten-type kinetic relationship was obtained which exhibited a $K_{0.5}$ value of about 100 μM and V_{max} values which increased with the amount of injected cRNA. The AMG dependence of transport obtained after coinjection of RS1-cRNA and SGLT1-cRNA at a molar ratio of 2 was significantly different. Thus, after coinjection, for AMG-transport expressed by different amounts of SGLT1-cRNA two apparent transport sites were obtained as has been described in kidney and intestine: One $K_{0.5}$ value was around 20 μM and the other around 1 mM [112]. If SGLT1-cRNA was injected with an excess of RS1-cRNA, the same AMG dependence of transport was measured as after injection of SGLT1-cRNA alone. The data suggest that Na^+-D-glucose cotransport systems contain RS1-type subunits in addition to the transporting SGLT1-type subunits. Figure 1 shows a model by which the role of SGLT1 and RS1 in Na^+-D-glucose cotransport may be explained. Thus, a Na^+-D-glucose cotransport system may be only composed of SGLT1-type subunits. Such systems may exist endogenously in the *Xenopus* oocytes and in fish (*see below*) or may be generated by expression of SGLT1 alone (Fig. 1*a*). In mammalian kidney and intestine or after coinjection of SGLT1-cRNA with a stoichiometric amount of RS1-cRNA into oocytes, RS1-polypeptides may associate with part of the SGLT1 polypeptides (Fig. 1*b*). The SGLT1/RS1-complexes may associate tightly with SGLT1 monomers. This association may result in cooperative interactions between the glucose transport sites, and high and low affinity glucose activation of transport may be distinguished. After coinjection of SGLT1-cRNA and excess RS1-cRNA, one RS1-polypeptide may associate per SGLT1 polypeptide (Fig. 1*c*). The association between the RS1/SGLT1 complexes may be weak and may not lead to cooperative interactions between the D-glucose binding sites. In this case, the glucose dependence of transport may be similar as after injection of SGLT1 alone.

In trying to determine the specificity of RS1, we investigated whether RS1 altered other endogeneous transport systems in *Xenopus* oocytes and performed coexpression experiments of RS1 with nonrelated transport proteins and with another Na^+-cotransporter from the SGLT1 family. No effects of RS1 on the endogeneous

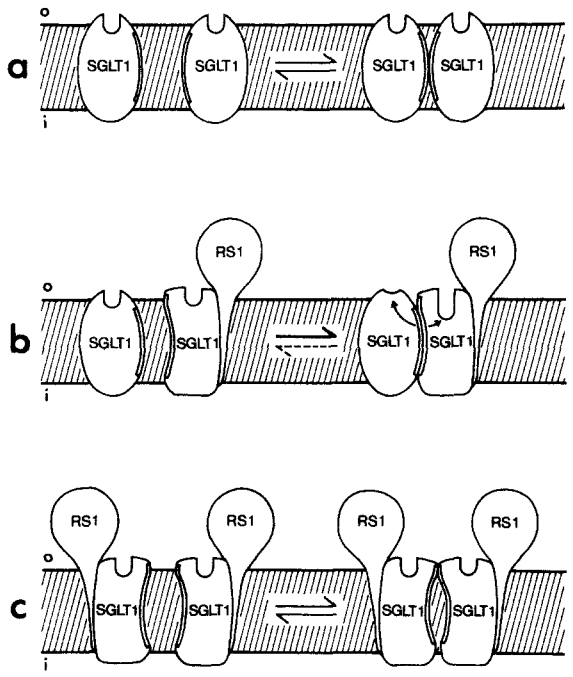

Fig. 1. Model for the interaction of expressed SGLT1- and RS1-type polypeptides. Na^+-D-glucose cotransport is expressed in *Xenopus* oocytes by injection of SGLT1-cRNA alone (*a*), by injection of stoichiometric amounts of SGLT1-cRNA and RS1-cRNA (*b*) or by injection of SGLT1-cRNA plus excess of RS1-cRNA (*c*). Double lines indicate interaction sites between the SGLT1 polypeptides. The extracellular and intracellular faces of the plasma membrane are labeled by *o* and *i*, respectively. D-glucose binding sites with high affinity (∪), intermediate affinity (∪) and low affinity (∪) are indicated.

activities of the (Na^+ + K^+) pump and the Na^+-L-glutamate cotransporter [97] were detected. Although many different ratios of coinjected cRNAs were tested, no effect of RS1 on expression of glucose transport by the facilitated diffusion transporter GLUT1 [69] and on expression of gamma-aminobutyric acid (GABA) transport by the Na^+-GABA cotransporter GAT1 [28] were observed. At variance, *myo*-inositol transport expressed by the SGLT1-homologous Na^+-*myo*-inositol cotransporter SMIT [56] was increased significantly when RS1-cRNA was coinjected at a molar ratio of 2 [112]. The species and tissue distribution of RS1-related cRNA was investigated by Northern blots and PCR experiments. RS1-homologous mRNAs were found in different mammals (rat, rabbit, pig, man) but not in *Xenopus* oocytes [112] and fish (A.I. Morrison, and H. Koepsell, *unpublished data*). RS1-homologous mRNAs were detected in renal outer cortex and outer medulla (pig, rabbit, man), in small intestine (pig, rabbit, sheep, man), in liver (pig), in spleen (pig) and in $LLCPK_1$ cells. No RS1-related mRNA could be detected in *Xenopus* oocytes, renal inner medulla, skeletal muscle, heart muscle and MDCK cells.

The data indicate that RS1 interacts specifically with SGLT1-type proteins which are components of Na^+-cotransport systems for sugars and other organic compounds. They suggest that Na^+-D-glucose cotransport systems in kidney and intestine contain RS1-homologous subunits which may have a regulatory function since (i) a monoclonal antibody against RS1 alters Na^+-dependent high affinity phlorizin binding which is specific for Na^+-D-glucose cotransport systems; (ii) RS1-homologous mRNA was observed in the localizations where Na^+-D-glucose cotransport has been described, and (iii) the biphasic glucose dependence of Na^+-D-glucose cotransport which is typical for kidney and has also been described in intestine was only observed by coexpression of SGLT1 and RS1 and not by expression of SGLT1 alone. Since it was found that RS1-homologous mRNAs are not present in inner renal medulla and in MDCK cells which contain the Na^+-*myo*-inositol cotransport protein SMIT, the interaction of RS1 with SMIT has to be explained by the structural similarity of SMIT to SGLT1 rather than indicating that the Na^+-*myo*-inositol cotransport system contains a RS1-type regulatory subunit. A similar observation has been made with the β-subunit of the (H^+ + K^+) ATPase which interacts with the α-subunit of the (Na^+ + K^+)-ATPase [75].

Since RS1-homologous mRNAs were found in liver and spleen where Na^+-D-glucose cotransport has not been described, RS1-type proteins may be components of SGLT1-type cotransporters for different substrates. Recent cloning experiments in intestine showed that RS1 is one of several homologous proteins. Thus, specific RS1-type proteins may be components of specific SGLT1-type transporters. The identification of the SGLT1 isoforms and RS1 isoforms which form specific Na^+-cotransport systems in different localizations is a challenging task for future investigations. The available functional and structural data on Na^+-D-glucose cotransport systems suggest that Na^+-D-glucose cotransport systems are hetero-oligomers which contain at minimum two SGLT1-type subunits and one or two RS1-type subunits. Whereas the SGLT1-type subunits are the transporting proteins, the RS1-type subunits may modulate the cooperative interactions of the SGLT1-subunits. It is also possible that RS1-type subunits play a role in the membrane insertion of the transporter as has been described for the β-subunit of the (Na^+ + K^+)-ATPase [63].

Many integral proteins which are capable of performing solute transport have been identified by expression cloning. Considering our data on the RS1-type subunit of the Na^+-D-glucose cotransport system, the question arises whether some of the other transport systems may also contain regulatory subunits. This idea is supported by recent reports on a hydrophilic protein which contains one hydrophobic α-helix at the N-ter-

minus and increases the expression of sodium independent uptake of neutral and dibasic amino acids in *Xenopus* oocytes [5, 118].

Conclusions

Taking the functional data on Na^+-D-glucose cotransport and on phlorizin binding, together with the data from target size analysis and with the genetic data, it appears to be probable that mammalian Na^+-D-glucose cotransport systems are hetero-oligomers containing two SGLT1-type and one or two RS1-type subunits. Different combinations of SGLT1-type subunits and RS1-type subunits may form the heterogeneous Na^+-D-glucose cotransport systems in kidney and intestine or other different Na^+-cotransport systems containing SGLT1-type transporting subunits. Crosstalk between different SGLT1-type Na^+-cotransport systems may occur if heterologous SGLT1-type transporting proteins exist within a Na^+-cotransport system. The role of the RS1-type subunits for the expression and regulation of functional transport systems has to be determined.

References

1. Alvarado, F. 1966. Transport of sugars and amino acids in the intestine: evidence for a common carrier. *Science* **151:**1010–1013
2. Aronson, P.S. 1978. Energy-dependence of phlorizin binding to isolated renal microvillus membranes. Evidence concerning the mechanism of coupling between the electrochemical Na^+ gradient and sugar transport. *J. Membrane Biol.* **42:**81–98
3. Barfuss, D.W., Schafer, J.A. 1981. Differences in active and passive glucose transport along the proximal nephron. *Am. J. Physiol.* **240:**F322–F332
4. Béliveau, R., Demeule, M., Ibnoul-Khatib, H., Bergeron, M., Beauregard, G., Potier, M. 1988. Radiation-inactivation studies on brush-border-membrane vesicles. General considerations, and application to the glucose and phosphate carriers. *Biochem. J.* **252:**807–813
5. Bertran, J., Werner, A., Moore, M.L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M., Murer, H. 1992. Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids. *Proc. Natl. Acad. Sci. USA* **89:**5601–5605
6. Birnir, B., Lee, H.-S., Hediger, M.A., Wright, E.M. 1990. Expression and characterization of the intestinal Na^+/glucose cotransporter in COS-7 cells. *Biochim. Biophys. Acta* **1048:**100–104
7. Blakely, R.D., Clark, J.A., Rudnick, G., Amara, S.G. 1991. Vaccinia-T7 RNA polymerase expression system: Evaluation for the expression cloning of plasma membrane transporters. *Anal. Biochem.* **194:**302–308
8. Blank, M.E., Bode, F., Baumann, K., Diedrich, D.F. 1989. Computer analysis reveals changes in renal Na^+-glucose cotransporter in diabetic rats. *Am. J. Physiol.* **257:**C385–C396
9. Blank, M.E., Bode, F., Huland, E., Diedrich, D.F., Baumann, K. 1985. Kinetic studies of D-glucose transport in renal brush-border membrane vesicles of streptozotocin-induced diabetic rats. *Biochim. Biophys. Acta* **844:**314–319
10. Brot-Laroche, E., Dao, M.T., Alcalde, A.I., Delhomme, B., Triadou, N., Alvarado, F. 1988. Independent modulation by food supply of two distinct sodium-activated D-glucose transport systems in the guinea pig jejunal brush-border membrane. *Proc. Natl. Acad. Sci. USA* **85:**6370–6373
11. Brot-Laroche, E., Serrano, M.-A., Delhomme, B., Alvarado, F. 1986. Temperature sensitivity and substrate specificity of two distinct Na^+-activated D-glucose transport systems in guinea pig jejunal brush-border membrane vesicles. *J. Biol. Chem.* **261:**6168–6176
12. Burckhardt, B., Kinne, R.K.H. 1992. Cotransporters and countertransporters. *In:* The Kidney: Physiology and Pathophysiology. D.W. Seldin and G. Giebisch, editors. pp. 537–586. Raven, New York
13. Charron M.J., Brosius, F.C., III, Alper, S.L., Lodish, H.F. 1989. A glucose transport protein expressed predominately in insulin-responsive tissues. *Proc. Natl. Acad. Sci. USA* **86:**2535–2539
14. Coady, M.J., Pajor, A.M., Wright, E.M. 1990. Sequence homologies among intestinal and renal Na^+/glucose cotransporters. *Am. J. Physiol.* **259:**C605–C610
15. Crane, R. K. 1960. Intestinal absorption of sugars. *Physiol. Rev.* **40:**789–825
16. Crane, R.K., Mandelstam, P. 1960. The active transport of sugars by various preparations of hamster intestine. *Biochim. Biophys. Acta* **45:**460–476
17. Csáky, T.Z., Thale, M. 1960. Effect of ionic environment on intestinal sugar transport. *J. Physiol.* **151:**59–65.
18. Debiec, H., Cross, H.S., Peterlik, M. 1991. 1,25-Dihydroxycholecalciferol-related Na^+/D-glucose transport in brush-border membrane vesicles from embryonic chick jejunum. Modulation by triiodothyronine. *Eur. J. Biochem.* **201:**709–713
19. Dorando, F.C., Crane, R.K. 1984. Studies of the kinetics of Na^+ gradient-coupled glucose transport as found in brush-border membrane vesicles from rabbit jejunum. *Biochim. Biophys. Acta* **772:**273–287
20. Elsas, L.J., Hillman, R.E., Patterson, J.H., Rosenberg, L.E. 1970. Renal and intestinal hexose transport in familial glucose-galactose malabsorption. *J. Clin. Invest.* **49:**576–585
21. Ferraris, R.P., Diamond, J. 1992. Crypt-villus site of glucose transporter induction by dietary carbohydrate in mouse intestine. *Am. J. Physiol.* **262:**G1069–G1073
22. Ferraris, R.P., Diamond, J.M. 1986. Use of phlorizin binding to demonstrate induction of intestinal glucose transporters. *J. Membrane Biol.* **94:**77–82
23. Ferraris, R.P., Diamond, J.M. 1986. A method for measuring apical glucose transporter site density in intact intestinal mucosa by means of phlorizin binding. *J. Membrane Biol.* **94:**65–75
24. Ferraris, R.P., Villenas, S.A., Hirayama, B.A., Diamond, J. 1992. Effect of diet on glucose transporter site density along the intestinal crypt-villus axis. *Am. J. Physiol.* **262:**G1060–G1068
25. Freeman, H.J., Quamme, G.A. 1986. Age-related changes in sodium-dependent glucose transport in rat small intestine. *Am. J. Physiol.* **251:**G208–G217
26. Freeman, T.C., Heavens, R.P., Dyer, J., Sirinathsinghji, D.J.S., Shirazi-Beechey, S.P. 1992. The expression of the Na^+/glucose cotransporter in the lamb small intestine. *Biochem. Soc. Trans.* **20:**1868
27. Gibbs, E.M., Hosang, M., Reber, B.F.X., Semenza, G., Diedrich, D.F. 1982. 4-azidophlorizin, a high affinity probe and photoaffinity label for the glucose transporter in brush border membranes. *Biochim. Biophys. Acta* **688:**547–556
28. Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S.,

Miedel, M.C., Davidson, N., Lester, H.A., Kanner, B.I. 1990. Cloning and expression of a rat brain GABA transporter. *Science* **249:**1303–1306

29. Haase, W., Heitmann, K., Friese, W., Ollig, D., Koepsell, H. 1990. Characterization and histochemical localization of the rat intestinal Na^+-D-glucose cotransporter by monoclonal antibodies. *Eur. J. Cell Biol.* **52:**297–309
30. Haase, W., Koepsell, H. 1989. Electron microscopic immunohistochemical localization of components of Na^+-cotransporters along the rat nephron. *Eur. J. Cell Biol.* **48:**360–374
31. Harig, J.M., Barry, J.A., Rajendran, V.M., Soergel, K.H., Ramaswamy, K. 1989. D-glucose and L-leucine transport by human intestinal brush-border membrane vesicles. *Am. J. Physiol.* **256:**G618–G623
32. Hazzard, C.E., Ahearn, G.A. 1992. Rapid stimulation of intestinal D-glucose transport in teleosts by 17α-methyltestosterone. *Am. J. Physiol.* **262:**R412–R418
33. Hediger, M.A., Budarf, M.L., Emanuel, B.S., Mohandas, T.K., Wright, E.M. 1989. Assignment of the human intestinal Na^+/glucose cotransporter gene (SGLT1) to the q11.2-qter region of chromosome 22. *Genomics* **4:**297–350
34. Hediger, M.A., Coady, M.J., Ikeda, T.S., Wright, E.M. 1987. Expression cloning and cDNA sequencing of the Na^+/glucose cotransporter. *Nature* **330:**379–381
35. Hediger, M.A., Ikeda, T., Coady, M., Gundersen, C.B., Wright, E.M. 1987. Expression of size-selected mRNA encoding the intestinal Na/glucose cotransporter in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA* **84:**2634–2637
36. Hediger, M.A., Mendlein, J., Lee, H.-S., Wright, E.M. 1991. Biosynthesis of the cloned intestinal Na^+/glucose cotransporter. *Biochim. Biophys. Acta* **1064:**360–364
37. Hediger, M.A., Turk, E., Wright, E.M. 1989. Homology of the human intestinal Na^+/glucose and *Escherichia coli* Na^+/proline cotransporters. *Proc. Natl. Acad. Sci. USA* **86:**5748–5752
38. Honold, K., Ludeke, B., Hengartner, H., Semenza, G. 1988. Stimulation of intestinal Na^+/D-glucose cotransport by monoclonal antibodies. *J. Membrane Biol.* **105:**165–175
39. Hopfer, U., Nelson, K., Perrotto, J., Isselbacher, K.J. 1973. Glucose transport in isolated brush border membrane from rat small intestine. *J. Biol. Chem.* **248:**25–32
40. Hopfer, U., Sigrist-Nelson, K., Groseclose, R. 1976. Jejunal and ileal D-glucose transport in isolated brush border membranes. *Biochim. Biophys. Acta* **426:**349–353
41. Hosang, M., Gibbs, E.M., Diedrich, D.F., Semenza, G. 1981. Photoaffinity labeling and identification of (a component of) the small-intestinal Na^+, D-glucose transporter using 4-azidophlorizin. *FEBS Lett.* **130:**244–248
42. Ikeda, T.S., Hwang, E.-S., Coady, M.J., Hirayama, B.A., Hediger, M.A., Wright, E.M. 1989. Characterization of a Na^+/glucose cotransporter cloned from rabbit small intestine. *J. Membrane Biol.* **110:**87–95
43. Jackowski, S., Alix, J.-H. 1990. Cloning, sequence and expression of the pantothenate permease (panF) gene of *Escherichia coli*. *J. Bacteriol.* **172:**3842–3848
44. Kanai, Y., Hediger, M.A. 1992. Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* **360:**467–471
45. Kaunitz, J.D., Gunther, R., Wright, E.M. 1982. Involvement of multiple sodium ions in intestinal D-glucose transport. *Proc. Natl. Acad. Sci. USA* **79:**2315–2318
46. Kaunitz, J.D., Wright, E.M. 1984. Kinetics of sodium D-glucose cotransport in bovine intestinal brush border vesicles. *J. Membrane Biol.* **79:**41–51
47. Kimmich, G.A. 1990. Membrane potentials and the mechanism of intestinal Na^+-dependent sugar transport. *J. Membrane Biol.* **114:**1–27
48. Kimmich, G.A., Randles, J. 1973. Interaction between Na^+-dependent transport systems for sugars and amino acids. Evidence against a role for the sodium gradient. *J. Membrane Biol.* **12:**47–68
49. Kimmich, G.A., Randles, J. 1980. Evidence for an intestinal Na^+: sugar transport coupling stoichiometry of 2.0. *Biochim. Biophys. Acta* **596:**439–444
50. Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M., Sachs, G. 1975. Sugar transport by renal plasma membrane vesicles. Characterization of the systems in the brush-border microvilli and basal-lateral plasma membranes. *J. Membrane Biol.* **21:**375–395
51. Koepsell, H., Fritzsch, G., Korn, K., Madrala, A. 1990. Two substrate sites in the renal Na^+-D-glucose cotransporter studied by model analysis of phlorizin binding and D-glucose transport measurements. *J. Membrane Biol.* **114:**113–132
52. Koepsell, H., Korn, K., Raszeja-Specht, A., Bernotat-Danielowski, S., Ollig, D. 1988. Monoclonal antibodies against the renal Na^+-D-glucose cotransporter. Identification of antigenic polypeptides and demonstration of functional coupling of different Na^+-cotransport systems. *J. Biol. Chem.* **263:**18419–18429
53. Koepsell, H., Madrala, A. 1987. Interaction of phlorizin with the Na^+-D-glucose cotransporter from intestine and kidney. *Top. Mol. Pharmacol.* **4:**169–202
54. Koepsell, H., Seibicke, S. 1990. Reconstitution and fractionation of renal brush border transport proteins. *Methods Enzymol.* **191:**583–605
55. Kong, C.-T., Yet, S.-F., Lever, J.E. 1993. Cloning and expression of a mammalian Na^+/amino acid cotransporter with sequence similarity to Na^+/glucose cotransporters. *J. Biol. Chem.* **268:**1509–1512
56. Kwon, H.M., Yamauchi, A., Uchida, S., Preston, A.S., Garcia-Perez, A., Burg, M.B., Handler, J.S. 1992. Cloning of the cDNA for a Na^+ *Imyo*-inositol cotransporter, a hypertonicity stress protein. *J. Biol. Chem.* **267:**6297–6301
57. Lescale-Matys, L., Dyer, J., Scott, D., Freeman, T.C., Wright, E.M., Shirazi-Beechey, S.P. 1993. Regulation of the ovine intestinal Na^+/glucose co-transporter (SGLT1) is dissociated from mRNA abundance. *Biochem. J.* **291:**435–440
58. Lin, J.-T., Szwarc, K., Kinne, R., Jung, C.Y. 1984. Structural state of the Na^+/D-glucose cotransporter in calf kidney brush-border membranes. Target size analysis of Na^+-dependent phlorizin binding and Na^+-dependent D-glucose transport. *Biochim. Biophys. Acta* **777:**210–208
59. Lindquist, B., Meeuwisse, G., Melin, K. 1962. Glucose-galactose malabsorption. *Lancet* **2:**666
60. Malo, C. 1988. Kinetic evidence for heterogeneity in Na^+-D-glucose cotransport systems in the normal human fetal small intestine. *Biochim. Biophys. Acta* **938:**181–188
61. Malo, C. 1990. Separation of two distinct Na^+/D-glucose cotransport systems in the human fetal jejunum by means of their differential specificity for 3-O-methylglucose. *Biochim. Biophys. Acta* **1022:**8–16
62. Malo, C., Berteloot, A. 1991. Analysis of kinetic data in transport studies: New insights from kinetic studies of Na^+-D-glucose cotransport in human intestinal brush-border membrane vesicles using a fast sampling, rapid filtration apparatus. *J. Membrane Biol.* **122:**127–141
63. McDonough, A.A., Geering, K., Farley, R.A. 1990. The sodium pump needs its β subunit. *FASEB J.* **4:**1598–1605
64. Meeuwisse, G.W., Dahlqvist, A. 1968. Glucose-galactose mal-

absorption. A study with biopsy of the small intestinal mucosa. *Acta Paediat. Scand.* **57:**273–280

65. Miyamoto, K.-I., Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y., Hagihira, H. 1991. Diabetes and glucose transporter gene expression in rat small intestine. *Biochem. Biophys. Res. Commun.* **181:**1110–1117
66. Moran, A., Turner, R.J., Handler, J.S. 1983. Regulation of sodium-coupled glucose transport by glucose in a cultured epithelium. *J. Biol. Chem.* **258:**15087–15090
67. Moran, A., Turner, R.J., Handler, J.S. 1984. Hexose regulation of sodium-hexose transport in LLC-PK_1 epithelia: The nature of the signal. *J. Membrane Biol.* **82:**59–65
68. Morrison, A.I., Panayotova-Heiermann, M., Feigl, G., Schölermann, B., Kinne, R.K.H. 1991. Sequence comparison of the sodium-D-glucose cotransport systems in rabbit renal and intestinal epithelia. *Biochim. Biophys. Acta* **1089:**121–123
69. Mueckler, M., Lodish, H.F. 1986. The human glucose transporter can insert posttranslationally into microsomes. *Cell* **44:**629–637
70. Murer, H., Hopfer, U. 1974. Demonstration of electrogenic Na^+-dependent D-glucose transport in intestinal brush border membranes. *Proc. Natl. Acad. Sci. USA* **71:**484–488
71. Murer, H., Sigrist-Nelson, K., Hopfer, U. 1975. On the mechanism of sugar and amino acid interaction in intestinal transport. *J. Biol. Chem.* **250:**7392–7396
72. Nakao, T., Yamato, I., Anraku, Y. 1987. Nucleotide sequence of *putP*, the proline carrier gene of *Escherichia coli* K12. *Mol. Gen. Genet.* **208:**70–75
73. Neeb, M., Fasold, H., Koepsell, H. 1985. Identification of the D-glucose binding polypeptide of the renal Na^+-D-glucose cotransporter with a covalently binding D-glucose analog. *FEBS Lett.* **182:**139–144
74. Neeb, M., Kunz, U., Koepsell, H. 1987. Identification of D-glucose-binding polypeptides which are components of the renal Na^+-D-glucose cotransporter. *J. Biol. Chem.* **262:**10718–10727
75. Noguchi, S., Maeda, M., Futai, M., Kawamura, M. 1992. Assembly of a hybrid from the α subunit of Na^+/K^+-ATPase and the β subunit of H^+/K^+-ATPase. *Biochem. Biophys. Res. Commun.* **182:**659–666
76. Ohta, T., Isselbacher, K.J., Rhoads, D.B. 1990. Regulation of glucose transporters in LLC-PK_1 cells: Effects of D-glucose and monosaccharides. *Mol. Cell. Biol.* **10:**6491–6499
77. Pajor, A.M., Wright, E.M. 1992. Cloning and functional expression of a mammalian Na^+/nucleoside cotransporter. *J. Biol. Chem.* **267:**3557–3560
78. Peerce, B.E., Wright, E.M. 1984. Sodium-induced conformational changes in the glucose transporter of intestinal brush borders. *J. Biol. Chem.* **259:**14105–14112
79. Peerce, B.E., Wright E.M. 1984. Conformational changes in the intestinal brush border sodium-glucose cotransporter labeled with fluorescein isothiocyanate. *Proc. Natl. Acad. Sci. USA* **81:**2223–2226
80. Pines, G., Danbolt, N.C., Bjørås, M., Zhang, Y., Bendahan, A., Eide, L., Koepsell, H., Storm-Mathisen, J., Seeberg, E., Kanner, B.I. 1992. Cloning and expression of a rat brain L-glutamate transporter. *Nature* **360:**464–467
81. Quamme, G.A., Freeman, H.J. 1987. Evidence for a high-affinity sodium-dependent D-glucose transport system in the kidney. *Am. J. Physiol.* **253:**F151–F157
82. Restrepo, D., Kimmich, G.A. 1986. Phlorizin binding to isolated enterocytes: Membrane potential and sodium dependence. *J. Membrane Biol.* **89:**269–280
83. Riklis, E., Quastel, J.H. 1958. Effects of cations on sugar absorption by isolated surviving guinea pig intestine. *Can. J. Biochem. Physiol.* **36:**347–362
84. Schloss, P., Mayser, W., Betz, H. 1992. Neurotransmitter transporters, a novel family of integral plasma membrane proteins. *FEBS Lett.* **307:**76–80
85. Schmidt, U.M., Eddy, B., Fraser, C.M., Venter, J.C., Semenza, G. 1983. Isolation of (a subunit of) the Na^+/D-glucose cotransporter(s) of rabbit intestinal brush border membranes using monoclonal antibodies. *FEBS Lett.* **161:**279–283
86. Scriver, C.R., Chesney, R.W., McInnes, R.R. 1976. Genetic aspects of renal tubular transport: Diversity and topology of carriers. *Kidney Int.* **9:**149–171
87. Semenza, G., Kessler, M., Hosang, M., Weber, J., Schmidt, U. 1984. Biochemistry of the Na^+, D-glucose cotransporter of the small-intestinal brush-border membrane. *Biochim. Biophys. Acta* **779:**343–379
88. Shirazi-Beechey, S.P., Hirayama, B.A., Wang, Y., Scott, D., Smith, M.W., Wright, E.M. 1991. Ontogenetic development of lamb intestinal sodium-glucose co-transporter is regulated by diet. *J. Physiol.* **437:**699–708
89. Silverman, M. 1976. Glucose transport in the kidney. *Biochim. Biophys. Acta* **457:**303–351
90. Silverman, M. 1980. Participation of the ring oxygen in sugar interaction with transporters at renal tubular surfaces. *Biochim. Biophys. Acta* **600:**502–512
91. Silverman, M. 1991. Structure and function of hexose transporters. *Annu. Rev. Biochem.* **60:**757–794
92. Silverman, M., Aganon, M.A., Chinard, F.P. 1970. Specificity of monosaccharide transport in dog kidney. *Am. J. Physiol.* **218(3):**743–750
93. Silverman, M., Turner, R.J. 1992. Glucose transport in the renal proximal tubule. *In:* Handbook of Physiology II. Section 8: Renal Physiology. E.E. Windhager, editor. pp. 2017–2036. Oxford University, New York
94. Smith, D.C., Hirayama, B.A., Wright, E.M. 1992. Baculovirus-mediated expression of the Na^+/glucose cotransporter in Sf9 cells. *Biochim. Biophys. Acta* **1104:**151–159
95. Smith, M.W., Turvey, A., Freeman, T.C. 1992. Appearance of phloridzin-sensitive glucose transport is not controlled at mRNA level in rabbit jejunal enterocytes. *Exp. Physiol.* **77:**525–528
96. Spangenberg, J., Veyhl, M., Poppe, R., Koepsell, H. 1993. Characterization of the regulatory subunit of the Na^+-D-glucose cotransporter. *Biol. Chem. Hoppe-Seyler* **374:**157
97. Steffgen, J., Koepsell, H., Schwarz, W. 1991. Endogeneous L-glutamate transport in oocytes of *Xenopus laevis*. *Biochim. Biophys. Acta* **1066:**14–20
98. Stevens, B.R., Fernandez, A., Hirayama, B., Wright, E.M. 1990. Intestinal brush border membrane Na^+/glucose cotransporter functions *in situ* as a homotetramer. *Proc. Natl. Acad. Sci. USA* **87:**1456–1460
99. Stewart, C.P., Turnberg, L.A. 1987. Glucose depolarizes villous but not crypt cell apical membrane potential difference: a micropuncture study of crypt-villus heterogeneity in the rat. *Biochim. Biophys. Acta* **902:**293–300
100. Storck, T., Schulte, S., Hofman, K., Stoffel, W. 1992. Structure, expression, and functional analysis of a Na^+-dependent glutamate/aspartate transporter from rat brain. *Proc. Natl. Acad. Sci. USA* **89:**10955–10959
101. Takahashi, M., Malathi, P., Preiser, H., Jung, C.Y. 1985. Radiation inactivation studies on the rabbit kidney sodium-dependent glucose transporter. *J. Biol. Chem.* **260:**10551–10556
102. Takata, K., Kasahara, T., Kasahara, M., Ezaki, O., Hirano, H. 1992. Immunohistochemical localization of Na^+-dependent glucose transporter in rat jejunum. *Cell Tissue Res.* **267:**3–9
103. Toggenburger, G., Kessler, M., Rothstein, A., Semenza, G.,

Tannenbaum, C. 1978. Similarity in effects of Na^+ gradients and membrane potentials on D-glucose transport by, and phlorizin binding to, vesicles derived from brush borders of rabbit intestinal mucosal cells. *J. Membrane Biol.* **40:**269–290

104. Turk, E., Zabel, B., Mundlos, S., Dyer, J., Wright, E.M. 1991. Glucose/galactose malabsorption caused by a defect in the Na^+/glucose cotransporter. *Nature* **350:**354–356
105. Turner, R.J., Kempner, E.S. 1982. Radiation inactivation studies of the renal brush-border membrane phlorizin-binding protein. *J. Biol. Chem.* **257:**10794–10797
106. Turner, R.J., Moran, A. 1982. Heterogeneity of sodium-dependent D-glucose transport sites along the proximal tubule: evidence from vesicle studies. *Am. J. Physiol.* **242:**F406–F414
107. Turner, R.J., Moran, A. 1982. Further studies of proximal tubular brush border membrane D-glucose transport heterogeneity. *J. Membrane Biol.* **70:**37–45
108. Turner, R.J., Moran, A. 1982. Stoichiometric studies of the renal outer cortical brush border membrane D-glucose transporter. *J. Membrane Biol.* **67:**73–80
109. Turner, R.J. 1983. Quantitative studies of cotransport systems: Models and vesicles. *J. Membrane Biol.* **76:**1–15
110. Ullrich, K.J., Rumrich, G., Klöss, S. 1974. Specificity and sodium dependence of the active sugar transport in the proximal convolution of the rat kidney. *Pfluegers Arch.* **351:**35–48
111. Veyhl, M., Püschel, B., Spangenberg, J., Dekel, C., Koepsell, H. 1992. Cloning of the β-subunit of the Na^+-D-glucose symporter. *FASEB J.* **6:**A1459
112. Veyhl, M., Spangenberg, J., Puschel, B., Poppe, R., Dekel, C., Fritzsch, G., Haase, W., Koepsell, H. 1993. Cloning of a membrane-associated protein which modifies activity and properties of the Na^+-D-glucose cotransporter. *J. Biol. Chem.* **268:** 25041–25053
113. Weber, W.-M., Püschel, B., Steffgen, J., Koepsell, H., Schwarz, W. 1991. Comparison of a Na^+-D-glucose cotransporter from rat intestine expressed in oocytes of *Xenopus laevis* with the endogenous cotransporter. *Biochim. Biophys. Acta* **1063:**73–80
114. Weber, W.-M., Schwarz, W. Passow, H. 1989. Endogenous D-glucose transport in oocytes of *Xenopus laevis*. *J. Membrane Biol.* **111:**93–102
115. Wells, R.G., Pajor, A.M., Kanai, Y., Turk, E., Wright, E.M., Hediger, M.A. 1992. Cloning of a human kidney cDNA with similarity to the sodium-glucose cotransporter. *Am. J. Physiol.* **263:**F459–F465
116. Werner, A., Moore, M.L., Mantei, N., Biber, J., Semenza, G., Murer, H. 1991. Cloning and expression of cDNA for a Na/Pi cotransport system of kidney cortex. *Proc. Natl. Acad. Sci. USA* **88:**9608–9612
117. Wu, J.-S.R., Lever, J.E. 1987. Monoclonal antibodies that bind the renal Na^+/glucose symport system. 1. Identification. *Biochemistry* **26:**5783–5790
118. Yan, N., Mosckovitz, R., Udenfriend, S., Tate, S.S. 1992. Distribution of mRNA of a Na^+-independent neutral amino acid transporter cloned from rat kidney and its expression in mammalian tissues and *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci USA* **89:**9982–9985
119. Ziegler, K., Frimmer, M., Fritzsch, G., Koepsell, H. 1990. Cyclosporin binding to a protein component of the renal Na^+-D-glucose cotransporter. *J. Biol. Chem.* **265:**3270–3277