Renal Na\textsuperscript{+}-glucose cotransporters

ERNEST M. WRIGHT

Department of Physiology, University of California Los Angeles School of Medicine, Los Angeles, California 90095-1751

Wright, Ernest M. Renal Na\textsuperscript{+}-glucose cotransporters. Am J Physiol Renal Physiol 280: F10–F18, 2001.—In humans, the kidneys filter ~180 g of D-glucose from plasma each day, and this is normally reabsorbed in the proximal tubules. Although the mechanism of reabsorption is well understood, Na\textsuperscript{+}-glucose cotransport across the brush-border membrane and facilitated diffusion across the basolateral membrane, questions remain about the identity of the genes responsible for cotransport across the brush border. Genetic studies suggest that two different genes regulate Na\textsuperscript{+}-glucose cotransport, and there is evidence from animal studies to suggest that the major bulk of sugar is reabsorbed in the convoluted proximal tubule by a low-affinity, high-capacity transporter and that the remainder is absorbed in the straight proximal tubule by a high-affinity, low-capacity transporter. There are at least three different candidates for these human renal Na\textsuperscript{+}-glucose cotransporters. This review will focus on the structure-function relationships of these three transporters, SGLT1, SGLT2, and SGLT3.

THE KIDNEYS PLAY A MAJOR ROLE in the regulation of plasma glucose levels, and ever increasing attention is now being given to renal glucose transporters as drug targets in the treatment of patients with diabetes mellitus. Each day, ~180 g of D-glucose are filtered from plasma by the kidneys, and this is all normally reabsorbed back into the blood in the proximal tubules. The model for glucose transport across the tubule is similar to that first proposed for the small intestine; i.e., glucose is first accumulated within the epithelium by a Na\textsuperscript{+}-glucose cotransporter (SGLT) in the brush-border membrane and then is transported out of the cell across the basolateral membrane by a facilitated sugar transporter (see Ref. 72).

There is compelling genetic evidence that there are two SGLTs in the human kidney (7, 8). Namely, patients with familial renal glycosuria (OMIM 233100; online, Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/OMIM) have no defect in intestinal glucose absorption, whereas patients with familial intestinal glucose-galactose malabsorption (OMIM 182380) only have a mild renal glycosuria. This suggests that the major intestinal Na\textsuperscript{+}/glucose transporter only plays a minor role in renal glucose reabsorption. Animal studies have supported this view. For example, in rabbit isolated perfused renal proximal tubules, the maximum rate of active glucose transport in the convoluted tubule (S1/S2) was an order of magnitude greater than that in the late straight tubule. The Michaelis-Menten coefficient value ($K_m$) for glucose was ~2 mM in S1/S2 and ~0.5 mM in S3 (1). This is consistent with sugar uptakes reported for human renal brush-border membrane vesicles (68), where there is some indication of high ($K_m$ ~0.5 mM)- and low ($K_m$ 6 mM)-affinity transporters.

In rabbit, the two SGLTs were spatially resolved by Turner and Moran (66, 67), who isolated brush-border membranes from the outer cortex (S1/S2) and outer medulla (S3). At 17°C with a Na\textsuperscript{+} gradient of 40 mM, the $K_m$ values were 6 mM in the outer cortex and 0.3 mM in the inner medulla. The high-affinity S3 transporter was more sensitive to phlorizin but more sensitive to competition by D-galactose than low-affinity S1/S2 transporter. Finally, Turner and Moran con-
cluded that the Na\(^+\)/glucose stoichiometry, as judged by indirect methods, was 1:1 for the S1/S2 and 2:1 for the S3 transporters. Although the kinetic heterogeneity of Na\(^+\)/glucose uptake by rabbit renal brush borders has been examined by others, alternative interpretations of the data have been put forward (41).

The presently accepted dogma is that the bulk of the filtered glucose is reabsorbed in the proximal convoluted tubule by a low-affinity, high-capacity SGLT, frequently referred to as “SGLT2,” and that the remainder is reabsorbed by the high-affinity cotransporter SGLT1.

SGLTs

**Protein**

Definitive identification of SGLTs came with our use of fluorescence group-specific reagents on rabbit intestinal brush-border membranes (51–55). This was based on our observation that isothiocyanates were potent irreversible blockers of cotransport and that this inhibition was prevented by the presence of Na\(^+\) and D-glucose. Nonspecific amino groups in brush borders were first labeled with phenyl isothiocyanate in the presence of Na\(^+\) and D-glucose, and then the transporters were labeled with fluorescein isothiocyanate in the absence of sugar. The SGLT was identified as a 75-kDa protein with a pI of 5.3, and additional studies with the fluorescent-labeled transporter demonstrated that Na\(^+\) opens up access to the sugar-binding site (see also Fig. 2).

**Genes**

In 1985, I set out to clone the intestinal SGLT but was initially unsuccessful because of our difficulty in obtaining protein microsequence information for synthesis of DNA probes. Then, along with Mike Coady, Tyson Ikeda, and Matthias Hediger, we developed a novel cloning strategy, expression cloning, to successfully isolate a cDNA coding for the rabbit high-affinity transporter (18, 19). When expressed in a variety of heterologous expression systems, this clone exhibits all the properties of the brush-border transporter, and there is clear evidence that this protein (SGLT1) is largely responsible for glucose and galactose transport across the intestinal brush border. Expression cloning soon became the preferred method for cloning new transporters, channels, and receptors and has been successfully exploited by my trainees (e.g., M. A. Hediger, A. M. Pajor, and B. A. Hagenbuch), colleagues, and others to clone new transporters and channels in the intestine, kidney, liver, and brain. This, and other methods (homology cloning), has resulted in the identification of three human SGLTs (SGLT1, SGLT2, and SGLT3) and over 55 other members of the SGLT1 gene family in bacteria, yeast, invertebrates, and vertebrates (see 65). These family members include the human myoinositol, iodide, and panthothenate and the bacterial proline, urea, and galactose cotransporters.

Table 1 lists the features of the three identified human SGLT genes. Human SGLT1 was first isolated from an intestinal cDNA library by using the rabbit SGLT1 as a probe (20) and was assigned to chromosome 22 q13.1 (17, 63). The gene spans 72 kb of genomic DNA on chromosome 22 (6, 64). Northern blot analysis revealed that the SGLT1 gene was transcribed in the human ileum and in rabbit small intestine, renal cortex, and outer renal medulla (42). SGLT2 was isolated from a human kidney library, mapped to chromosome 16 p11.2, and this gene is transcribed in human kidney cortex, and, to a much lesser degree, in the ileum (70, 71). Rat kidney SGLT1 and SGLT2 genes are transcribed in the cortex and the outer medulla, respectively (74). SGLT3 was isolated from a pig renal cell line by using our rabbit SGLT1 probe, and it was designated SAAT1 on the basis of expression experiments in oocytes, where it produced a modest stimulation of amino acid transport uptake (29). We subsequently demonstrated that it was a low-affinity glucose (not galactose)-Na\(^+\) cotransporter and referred to it initially as pig SGLT2 (37, 38). The human isofrom was also identified on chromosome 22 (6). Interestingly, SGLT3 is located downstream of SGLT1 (within 0.15 mb) and has a similar intron-exon organization, suggesting an ancient gene duplication. Pig SGLT3 is transcribed in kidney, intestine, liver, and spleen (29).

There is little direct information about the translation of SGLT mRNAs in the human kidney, because of the lack of suitable antibodies to discriminate among the three proteins. Several polyclonal antipeptide antibodies have been raised against SGLT1 that do recognize proteins in brush borders of the rodent cortical tubules and small intestine (3, 24, 25, 61). However, these antibodies may also recognize other SGLTs because there is close similarity between the peptide sequences used to raise the antibodies. Progress requires the development of specific antibodies to each of the isoforms.

**Orphan Clones**

In addition to the SGLTs of known function, there is at least one SGLT human gene whose function is unknown (6), and at least six orphan cDNAs have been isolated from rabbit and human kidney libraries (42 and M. G. Martin and E. M. Wright, unpublished
The chromosome 22 SGLT-related gene AP000362.1 (6) is reported to code for a 249-residue polypeptide with similarity to SGLT2. Close inspection suggests that exons 2, 3, and 5 code for peptides with significant homologies to the corresponding SGLT 1–3 sequences. However, other exons appear to be missing. Given that chromosome 22 comprises <2% of the genomic DNA and that the SGLT1 gene family is quite large, it is to be expected that more SGLT1 genes will emerge from the genome project. Only one SGLT1 family member gene, SMIT, was found on chromosome 21 (15). The Sanger Centre has discovered a new member of the SGLT family during the sequencing of chromosome 1 (gene dJ1024N4, http://webace.sanger.ac.uk. accession CAC00574. GI: 9588428). Inspection of the genomic sequence (E. Turk and E. M. Wright, unpublished observations) shows that this SGLT1-related gene, at position 1p32.1-33, has 14 exons spanning 20 kb of genomic DNA. There is one less exon than SGLT1 and SGLT3, due to the elimination of the intron corresponding to the one between exons 4 and 5 in SGLT1. The gene codes for a protein of 674 residues with high similarity to SGLT1. As yet, there is no information available about either the tissue distribution of the transcript or the function of the protein. The expression and function of SGLT-related genes in the kidney and other tissues will continue to be an active area of investigation.

**SGLT Proteins**

The SGLT1–3 genes code for proteins of 659–672 residues, with a predicted mass of 73 kDa. Amino acid sequence alignments show that SGLT3 and SGLT2 have identities of 70 and 59% to SGLT1. The secondary structure model for SGLT1 is shown in Fig. 1, and, although there have been few or no experimental studies, SGLT2 or SGLT3 is predicted to have the same secondary structure profile. The model contains 14 transmembrane a-helices (TMH) with both the hydrophobic NH2 terminus and the COOH terminus of TMH 14 facing the extracellular solution. All probably use the consensus N-linked glycosylation site (N245) between TMH 6 and 7. The model is based on 1) experimental studies on SGLT1 (see 65); 2) experimental studies on related SGLT1 family members (Na+/iodide, 32; Na+/proline, 26, 69); and 3) a computational analysis of SGLT1, SGLT2, and pig SGLT3 using algorithms such as PredictProtein and Memsat (65).

On the basis of freeze-fracture electron microscopic examination of recombinant membrane proteins expressed in *Xenopus laevis* oocytes, we have established that SGLT1 functions as a monomer (11, 75). The

![Fig. 1. The amino acid sequence and secondary structure of human (h) Na\(^{+}\)-glucose cotransporter SGLT1. The 664-residue protein is shown as a pearl chain, with each residue indicated by a single-letter code. The secondary structure model shows 14 transmembrane helices (TMH) with both the NH2 and COOH termini facing the extracellular solution (65).](http://ajprenal.physiology.org/)

---

**hSGLT1**

<table>
<thead>
<tr>
<th>TMH 1</th>
<th>TMH 2</th>
<th>TMH 3</th>
<th>TMH 4</th>
<th>TMH 5</th>
<th>TMH 6</th>
<th>TMH 7</th>
<th>TMH 8</th>
<th>TMH 9</th>
<th>TMH 10</th>
<th>TMH 11</th>
<th>TMH 12</th>
<th>TMH 13</th>
<th>TMH 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EXTRACELLULAR**

**CYTOPLASM**

---

[Figure 1](http://ajprenal.physiology.org/) shows the amino acid sequence and secondary structure of human (h) Na\(^{+}\)-glucose cotransporter SGLT1. The 664-residue protein is shown as a pearl chain, with each residue indicated by a single-letter code. The secondary structure model shows 14 transmembrane helices (TMH) with both the NH2 and COOH termini facing the extracellular solution (65).
density of intramembrane particles in the plasma membrane of oocytes expressing SGLT1 is directly proportional to SGLT1 transport activity, and the area of the particle in the membrane is consistent with TMH 14. The relationship between area and number of TMHs was established by expressing membrane proteins of known structure in oocytes and comparing their area with those obtained from two-dimensional projection maps. We also concluded that the 14 TMHs are arranged in an elliptical array with an x-to-y ratio of 1.2. This quaternary structure was confirmed in a recent study of a purified SGLT protein reconstituted into liposomes (62). These results are in contrast to our previous irradiation inactivation experiments on rabbit brush-border membranes, where the rate of transport decreased with increasing radiation dose with an apparent size of a 290-kDa homotetramer (60). However, radiation decreased the size of SGLT1 protein (detected on Western blots) with an apparent size of 70 kDa. The explanation for the higher estimate (290 kDa) by irradiation inactivation is not clear, but similar discrepancies have been reported for other transporters.

The only information available about the packing arrangement of the 14 TMHs in these proteins comes from cross-linking on the bacterial homolog vSGLT (73). In these experiments, vSGLT was split into two halves, the NH2- and COOH-terminal halves, and each half was expressed in bacterial cells. The split proteins were only functional when expressed in the same cell, but the proteins migrated on SDS-PAGE gels at one-half the expected mobility of the full-length transporter. However, when each half contained a single cysteine residue (Cys149 and Cys423), the proteins migrated as the full-length protein after cross-linking with bis-maleimidoethane or iodine. We conclude TMH 4 and 5 lie in close proximity to TMH 10 and 11 (<8 Å). Further studies are in progress to obtain a TMH helical packing map, as has already been done for lactose permease (see 27). Additional studies (see STRUCTURE AND FUNCTION) indicate that TMH 10–13 lie in close proximity to form the sugar translocation pathway through SGLT1.

TRANSPORT ACTIVITY OF SGLTs

Although the SGLT clones have been expressed in a number of heterologous expression systems ranging from insect to primate cells (e.g., 2, 59), the most complete characterizations have been carried out in X. laevis oocytes. A summary of the salient features is presented in Table 2. There are no functional studies on human SGLT3,1 so the features of SGLT3 refer to work on the pig clone. At least with SGLT1 and SGLT2, there is a fair convergence of kinetic parameters among different species (human, rabbit, rat; 23, 28, 74). The reader should be aware that SGLT2, unlike SGLT1 and 3, expresses very poorly in oocytes and COS-7 cells; e.g., in SGLT2 oocytes, the Na+-dependent uptake of sugar and the Na+/sugar currents were, at most, only 2–4 times greater than those in control oocytes (28), whereas the sugar fluxes and currents in SGLT1 and SGLT3 oocytes were normally 500–2,000 times greater than those in controls. This means that the reported SGLT2 kinetics parameters are subject to uncertainty.

All three SGLTs transport α-methyl-D-glucoside (α-MDG), a nonmetabolized, model substrate, in a sodium-dependent manner with apparent affinity (K0.5) values from 0.4 (SGLT1) to 2 mM (SGLT2 and SGLT3). For D-glucose the K0.5 values are 0.4, 2, and 6 mM, respectively. Although SGLT1 does not discriminate among α-MDG, glucose, and galactose, neither SGLT2 nor SGLT3 transports D-galactose efficiently (K0.5 > 20 mM). It appears the S1/S2 transporter in rabbit kidney does not transport D-galactose efficiently either (see the beginning of this review). Another model substrate for SGLT1, 3-O-methyl-D-glucose, is also not transported by either SGLT2 or 3. Phlorizin is a potent competitive inhibitor of all the transporters, with inhibitor constants (Ki) of 0.2 mM for SGLT1 and 10 μM for SGLT3, and an apparent Ki of 1 μM for SGLT2. This variation in Ki between transporters is no greater than that among the species for SGLT1, with Ki values ranging from 0.01 μM in rat to 0.75 μM in rabbit (23).

The apparent sodium affinities for SGLT1 and 3 are voltage dependent, but at hyperpolarizing membrane potentials (~150 mV) the K0.5 asymptotes to ~3 mM (23, 37, 38). The Na+ K0.5 for SGLT2 is thought to be >100 mM (28, 74). The stoichiometry of Na+ to sugar transport, when measured directly with radioactive fluxes under voltage-clamp conditions, is two for both SGLT1 and 3 (4, 39). Indirect methods were used in the case of SGLT2, but, given the signal-to-noise problems with both the rat and human clones, there is uncertainty in the reported 1:1 stoichiometry (28, 74). Other cations such as H+ and Li+ can drive sugar transport through SGLT1 and SGLT3 (22, 38), but this has not been tested for SGLT2.

---

1Preliminary functional studies of human SGLT3 in oocytes indicate that both the selectivity and kinetics are similar to those reported for pig SGLT3 (C. Volk, A. Diez-Sampedro, B. A. Hirayama, E. M. Wright, and H. Koepsell, unpublished observations).

### Table 2. Transport properties of SGLTs

<table>
<thead>
<tr>
<th></th>
<th>SGLT1</th>
<th>SGLT2</th>
<th>SGLT3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0.5 (d-glucose; mM)</td>
<td>0.4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>K0.5 (Na+; mM)</td>
<td>3</td>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>Coupling (Na+/glucose)</td>
<td>2</td>
<td>(1)</td>
<td>2</td>
</tr>
<tr>
<td>Turnover, s−1</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Phlorizin K, μM</td>
<td>0.22</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Sugar selectivity</td>
<td>d-glc ~ d-gal</td>
<td>d-glc &gt; d-gal</td>
<td>d-glc &gt; d-gal</td>
</tr>
<tr>
<td>Na+ uniport</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Water cotransport</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

K0.5, affinity value; glc, glucose; gal, galactose. *Properties of pig SGLT3. Those for SGLT1 and SGLT2 are for human. †Determined at ~150 mV, but the voltage for SGLT2 was not specified. ‡Determined using Na+ and sugar fluxes under voltage clamp, except for SGLT2. §Taken from the maximum rate of Na+/glucose cotransport at saturating voltages (~150 mV) and the number of transporters estimated from SGLT charge movements (Qmax).
In summary, the major differences in function between SGLT1 on one hand and SGLT2/SGLT3 on the other relate to differences in the selectivity for sugar transport. SGLT1 accepts the natural sugars D-glucose and D-galactose with comparable affinities and a variety of synthetic sugars such as α-MDG and 3-O-methyl-D-glucose. SGLT2 and 3 are much more restrictive in that they prefer D-glucose to D-galactose or 3-O-methyl-D-glucose. The affinity of SGLT1 for D-glucose is 10-fold higher than for SGLT2 or 3. The Na⁺/glucose coupling ratio is two for SGLT1 and SGLT3, and there is a hint that it is one for SGLT2. All three are quite sensitive to the specific inhibitor phlorizin.

**TRANSPORT MECHANISMS**

The kinetics of rabbit, rat, and human SGLT1 transport has been examined in considerable detail by measuring steady-state and pre-steady-state methods (16, 22, 23, 34, 35, 39, 43–50b). A limited series of experiments has also been conducted on pig SGLT3 (37, 38), but there is little information available about SGLT2, owing to its low level of expression (see TRANSPORT ACTIVITY OF SGLTS). However, the similarity in kinetics of SGLT1, SGLT3, and other members of the SGLT1 family of proteins [SMIT (14) and NIS (9)] points to a common transport mechanism.

A six-state ordered kinetic model with “mirror” symmetry is shown in Fig. 2A. It is assumed that the carrier is divalent and that two external Na ions bind in a single reaction step before sugar (or phlorizin) to neutralize this charge. There are two transport loops linking the six discrete states of the carrier: one carries out the transport of Na ions in the absence of sugar, and the other carries out the coupled transport of two Na ions and one sugar molecule. Of the three transmembrane state transitions, there is only one charge translocation step (1–6); thus SGLT1 currents are carried by state transitions of the unloaded carrier. Rapid equilibrium was not assumed, and a formal description of the reaction scheme has been obtained. We have carried out extensive “iterative” numerical simulations of both our pre-steady-state and steady-state data and have arrived at a set of rate constants that account for the global properties of the transporter (see 16, 33, 50b).

Substantial experimental evidence has been gathered in favor of this model. For example, 1) the coupling coefficient for Na⁺ and glucose transport is 2.1; 2) in the absence of sugar, Na⁺ is transported with kinetics similar to those of Na⁺-glucose cotransport; i.e., two Na ions are transported per cycle; 3) the maximum rate of Na⁺-glucose cotransport at saturating membrane potentials is independent of sodium concentration; and 4) rapid voltage-jump experiments in the absence of sugar show SGLT1 pre-steady-state currents that represent the redistribution of the states of the transporter between C2 and C6. The cotransport turnover time is ~60 s at 22°C, and, for the Na⁺ uniport, is ~10 s. The slow steps in the cycle appear to be the internal dissociation of Na⁺ from the carrier (C5–C6) and the conversion of C6 to C1, but which of these is the rate-limiting step depends critically on the membrane potential. Recently, two independent studies using the giant excised-patch technique (10, 58) have provided additional support by showing SGLT1 can work in the reverse mode (transporting sugar from cytoplasm to the exterior). As predicted by our model, the affinities for sugar and Na⁺ on the cytoplasmic face of the membrane are orders of magnitude lower than those on the outward face of the membrane.

Berteloot and colleagues (12) have critically examined our six-state model and have pointed out that we have oversimplified the reaction scheme by assuming Na⁺ binding can be described as a single reaction (either the binding of the first Na⁺ is much faster than the second or the two Na⁺ bind with identical rate constants to similar sites; 44). According to Berteloot et al. (12), this may only be valid over a restricted range
of Na\(^+\) concentrations or when there is very strong positive cooperativity between the Na\(^+\)-binding sites. Evidence that there is strong interaction between the two Na\(^+\)-binding sites comes from the near identity of the Hill coefficient and the coupling coefficient (39). On balance, the gain in simplifying the model from 20 to 14 rate constants outweighs the more rigorous approach.

SGLT1 and other cotransporters also behave as channels and pumps for urea and water (31, 35, 36, 40), but this is beyond the scope of the present review.

**STRUCTURE AND FUNCTION**

The present hypothesis is that Na\(^+\) and sugar have two separate pathways through SGLT1: Na\(^+\) permeates through the NH\(_2\)-terminal half of the protein, and sugar permeates through the COOH-terminal half (Fig. 3). Na\(^+\) binding to the NH\(_2\)-terminal domain then causes a long-range conformational change in the protein to permit sugar binding and translocation. The experimental basis for this hypothesis comes from studies of SGLT1/SGLT3 chimeras that suggested that the COOH-terminal domain (residues 407–662 containing TMHs 10–14) determined sugar affinity and selectivity (47). This was reinforced by experiments showing that the truncated protein containing residues 407–662 (C5) behaved as a sugar uniporter either when expressed in oocytes or when reconstituted into proteoliposomes (45, 46). We speculate that TMHs 10–13 form the sugar translocation pathway through SGLT1 (Fig. 2B), as TMH 14 is missing from some members of the SGLT1 family and deletion of this TMH does not eliminate sugar transport. Although we have not yet been able to obtain full Na\(^+\)-glucose co-transport activity by coexpressing separate N9 and C5 cRNAs in oocytes, we have been successful in expressing a split Vibrio transporter (vSGLT) in bacteria (73).

A common feature of SGLT1 with cysteines at 457, 468, and 499 is that they are only sensitive to MTS reagents when the transporter is in the C2 conformation (Fig. 2A). MTS accessibility to these residues is blocked when the reaction is carried out 1) in the absence of Na\(^+\), 2) in the presence of Na\(^+\) and either glucose or phlorizin, and 3) in the presence of Na\(^+\) when the membrane potential is depolarized. The access of MTS reagents to the residues is directly proportional to the probability of the protein being in the C2 state. One interpretation of these observations is that residues 457, 468, and 499 line the sugar translocation pathway through the transporters and that this pathway is only accessible in the C2 conformation (Fig. 2B).

Given the dimensions of the MTS reagents that can gain access, phlorizin that can block access, and the
sugar analogs that can be transported, we suggest that sodium produces a large change in the tilt or rotation of one or more TMHs in the 10–13 helical bundle (Fig. 2B). Direct evidence for motion of 457C during the C1-C2 transitions comes from monitoring fluorescence of rhodamine-labeled protein during voltage-jump experiments (35). Both the level and time course of the change in fluorescence closely followed the charge movements that are a hallmark of the voltage-induced changes in state of the protein. We further suggest that sugar translocation to the cell interior requires further large changes in helical tilt and/or rotation and that it is these changes in conformation that account for the cotransport of water and urea (2 Na\textsuperscript+ :1 glucose:250 water; 31, 36, 40). Presently, we are extending these cysteine-scanning experiments to map the conformational changes for the cotransporter during other state transitions and to determine the TMH packing.

We postulate that the structural basis for cotransport is similar among the SGLTs (SGLT1–3) and among the other members of the large SGLT superfamily.

**IDENTITY OF RENAL SGLTs**

A question still to be resolved is the identity of the genes responsible for glucose reabsorption across the brush border of the human proximal tubule. Although it is clear from genetic studies that SGLT1 plays only a minor role, perhaps in the late tubule, the identity of the low-affinity renal SGLT (“SGLT2”) is still unclear. There are at least two low-affinity SGLTs, SGLT2 and SGLT3, and there are likely to be others. Another SGLT is indicated by the results of the chromosome 22 sequencing project (6), and our screening of human renal cDNA libraries has identified four other clones (M. G. Martin and E. M. Wright, unpublished observations). A similar problem exists in rodent models, and the information that does exist about the expression of SGLTs, e.g., SGLT1 and SGLT2 in the rat kidney (74), only relates to mRNA levels. In the absence of information about SGLT protein in renal brush borders, it is difficult to draw definitive conclusions, especially when there are examples where there is a divergence between SGLT mRNA and protein levels (30). One powerful approach with animal models is to use gene-knockout technology to explore the physiological role of SGLTs, similar to that employed already with the basolateral facilitated transporter GLUT-2 (13). GLUT-2-null mice show a severe renal glycosuria. However, extrapolation of results from animal models to humans may not always be justified. One useful strategy with humans is to determine which SGLT gene is responsible for familial renal glycosuria, but this may not be foolproof as it has already been reported that mutations in GLUT2 may cause renal glycosuria in patients with the Fanconi-Bickel syndrome (57). Finally, it should be noted that in a linkage study of 25 patients in 5 unrelated families with renal glycosuria (3a), the gene for the renal glycosuria (GLYS1, OMIM 233100) was segregated with the Human Leukocyte Antigen complex. This suggests that GLYS1 is on chromosome 6p21.3, but so far the gene has not been isolated. However, I do anticipate that the gene coding for the major renal bush-border Na\textsuperscript+ -glucose cotransporter will soon be positively identified, and this will open a new era in the study of the renal handling of sugars.

I thank Debra Moorehead, Matthias Quick, and Bruce Hirayama for assistance with the figures.

The studies reported from this laboratory were made possible by the work of many talented colleagues, postdoctoral fellows, students, and research assistants, and by the financial support of National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-19567, DK-44602, and DK-44582.

**REFERENCES**


