Review

Sodium-Coupled Neurotransmitter Transporters

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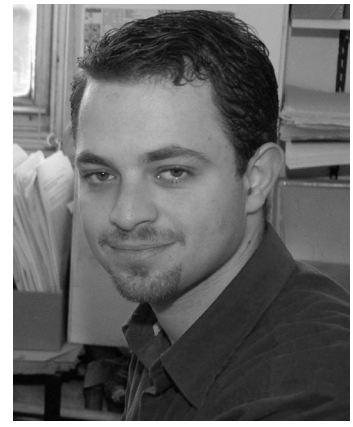

Elia N. Zomot was born on March 18, 1980. He earned his B.Sc. degree in biology from Hebrew University Life Sciences, Jerusalem, Israel, in 2000, his M.Sc. degree in biochemistry from Hebrew University, Hadassah Medical School, Jerusalem, Israel, in 2003, and his Ph.D. degree will be completed in biochemistry from Hebrew University, Hadassah Medical School, Jerusalem, Israel, in 2008.

1. Introduction

Sodium-coupled neurotransmitter transporters are located in the plasma membranes of neurons and glia, where they are present at high density in those areas of the cell membrane that face the synapse (Figure 1). They serve to keep the extracellular neurotransmitter concentrations sufficiently low, so that the postsynaptic receptors are able to detect signaling by the presynaptic nerve cell in the form of exocytotically released transmitters (Figure 1). Thus, neurotransmitter transporters are key elements in the termination of the synaptic actions of neurotransmitters. Moreover, they serve to keep the extracellular transmitter concentrations below neurotoxic levels. Termination of synaptic transmission by transporters takes place with most neurotransmitters, including L-glutamate, γ-aminobutyric acid (GABA), glycine, dopamine, serotonin, and norepinephrine. Another termination mechanism is observed with cholinergic transmission. After dissociation from its receptor, acetylcholine is hydrolyzed into choline and acetate. Even then, the choline moiety is subsequently recovered by sodium-dependent transport. Because the concentration of the transmitters in the nerve terminals is orders of magnitude higher than that in the synaptic cleft, energy input is required to move the neurotransmitters against their concentration gradients. The transporters, located

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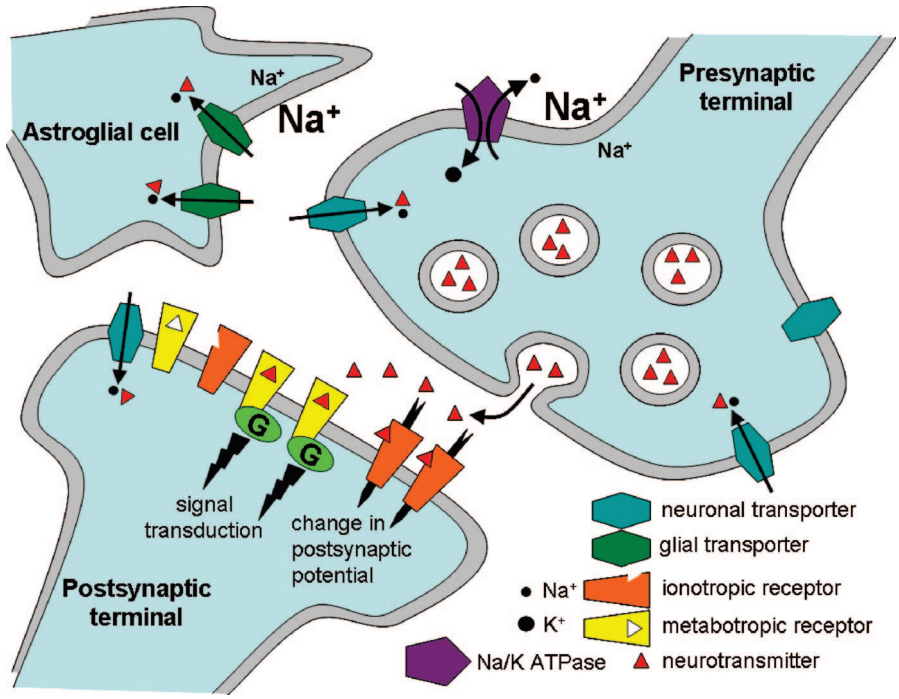

Figure 1. Neurotransmission at a neuronal synapse. Illustration depicts a typical synapse where the neurotransmitter (such as glutamate or GABA) (red triangle) stored in presynaptic vesicles fuses with the presynaptic membrane, is released at the synaptic cleft, and activates ionotropic (orange trapezoids) or metabotropic (yellow trapezoids) receptors found on the postsynaptic cell membrane. This results in a change in membrane potential by opening or closing ion channels either directly (ionotropic receptors) or after signal transduction (metabotropic receptors). The neurotransmitter is cleared from the synaptic cleft by the action of neurotransmitter transporters that translocate it into astroglial, the presynaptic, or the postsynaptic cell. These transporters can be found on neurons (blue hexagons) or on astroglial cells (green hexagons). Uptake of the neurotransmitter into these cells is achieved by cotransport with sodium ions (small black circles) by exploiting the electrochemical gradient of sodium, generated by the Na^+/K^+-ATPase (purple pentagon), to drive the energy-consuming intracellular accumulation of the neurotransmitter.

in the plasma membranes of nerve endings and glial cells, obtain this energy by coupling the flow of neurotransmitters to that of sodium. The (Na^+ plus K^+)-ATPase generates an inwardly directed electrochemical sodium gradient, which is used by the transporters to drive "uphill" transport of the neurotransmitters (reviewed in refs 1–4). Neurotransmitter uptake systems have been investigated in detail using plasma membranes obtained upon osmotic shock of synaptosomes, and these studies show that these transporters couple the flow of neurotransmitters not only to that of sodium but also to that of additional ions, such as potassium or chloride.[1–3]

One of the major pieces of evidence for the importance of the transporters comes from the study of knockout mice. In the dopamine transporter knockout mice, the decay of extracellular dopamine is about 100 times longer than normal.[5] The study of glutamate transporter knockout mice indicates that glutamate transporters, in particular GLT-1,[6] play a central role in preventing both hyperexcitability and excitotoxicity.[7] The transporters are slow, with turnover rates ranging from less than 1 up to 15 s^{-1}. Thus, a single transporter requires at least 60 ms to complete its cycle. On the other hand, transmitter clearance at fast synapses occurs within a few milliseconds. How can this apparent contradiction be resolved? Often the rate-limiting step is a slow conformational change during one of the steps of the translocation cycle. For instance, in the case of the GABA transporter GAT-1, this is a slow charge-moving conformational change following sodium binding,[8,9] and once the transporters are in the sodium-bound state, neurotransmitter binding can be very fast. However, in this scenario, one would then need a density of transporters at the synaptic sites in the order of 10^3 μm^{-2} of membrane (discussed in ref 10). Such densities and sometimes up to 10-fold higher have indeed been observed at relevant locations in the brain.[11,12]

Sodium-coupled neurotransmitter transporters are of considerable medical interest. Because they function to regulate neurotransmitter activity by removing the transmitters from the cleft, specific transporter inhibitors can be potentially used as novel drugs for neurological disease. For instance, attenuation of GABA removal will prolong the effect of this inhibitory transmitter. Thus, inhibitors of GABA transport could represent a novel class of antiepileptic drugs. Well-known inhibitors, which block the biogenic amine transporters, include antidepressant drugs, such as Prozac, and stimulants, such as amphetamines and cocaine. At excessive local concentrations, the neurotransmitter glutamate causes cell death by activating *N*-methyl-D-aspartic acid receptors, resulting in calcium entry. This transmitter has been implicated in neuronal destruction during ischemia, epilepsy, stroke, amyotropic lateral sclerosis, and Huntington's disease. Therefore, glutamate transporters are key players in preventing glutamate from acting as an excitotoxin.

The recently elucidated structures of bacterial and archeal homologues of the neurotransmitter transporters[13–15] have provided new insights on the structural basis of the function of real neurotransmitter transporters, and this aspect is the focus of this review.

1.1. Brief History of Two Neurotransmitter Transporter Families

After the purification of a GABA transporter from rat brain in a functional form,[16,17] protein sequence information obtained

from it led to the cloning of the first neurotransmitter transporter, GAT-1.[18] Subsequently, the expression cloning of a norepinephrine transporter[19] provided the first evidence that these two proteins are the first two members of a novel superfamily of neurotransmitter transporters, termed SLC6 or NSS (neurotransmitter:sodium symporters). Using the polymerase chain reaction and other technologies relying on sequence information, other members of this family were rapidly cloned (reviewed in refs 20 and 21). Besides transporters for GABA and norepinephrine, other members include transporters for dopamine, serotonin, and glycine. This family also includes transporters of amino acids, which are not neurotransmitters, and also bacterial homologues, but the neurotransmitter transporters of this family have in common that they transport the neurotransmitter not only together with sodium but also with chloride. Transporters for glutamate, the major excitatory neurotransmitter in the brain, were not identified using the homology screening approach, because they belong to a distinct family, termed SLC1. Therefore, successful cloning of its first members was achieved using different approaches.[6,22,23] Glutamate is also transported together with sodium ions. The process does not depend upon chloride but involves potassium transport in the opposite direction. As will be discussed later, the structures of the two types of neurotransmitter transporters are very different from each other. The resolution of the structure of the SLC6 homologue LeuT, a bacterial leucine transporter,[14] is much higher than that of the glutamate transporter homologue Glt_{Ph}.[13] Consequently, our insights into the structural basis of the mechanism of transport are more advanced in the SLC6 transporters than their SLC1 counterparts, as will become apparent in this review.

1.2. Alternating Access Mechanism

Generally, transporters function by exposing their binding sites alternately to either side of the membrane, catching up their cargo on one side and releasing it on the other. A widely accepted theory proposes that this can be accomplished using two gates, with only one open at a time, just like locks in a waterway.[24] Support for this idea comes from crystal structures of transporters, which invariably show a cavity in the transporter closed off from the aqueous space on either or both sides of the membrane. These structures also show that the size of these gates can be quite substantial.[14,25] The binding pocket of ion-coupled transporters also has binding sites for the ion(s) that power(s) the transport process. Therefore, the ion(s) and the substrate are transported together such that the energy released as the ion moves down its gradient is used to power the uphill movement of the substrate. A question yet to be resolved at the molecular level is how the "driving" ion and the "driven" substrate move through an ion-coupled transporter. Conceptually, a sodium-driven neurotransmitter transport cycle is depicted in steps 1–4 in Figure 2A. The outward-facing transporter (external gate open) binds one or more sodium ions and the neurotransmitter (step 1). As a consequence, the external gate closes, and this closure is somehow coupled to the opening of the internal gate (step 2). This coupling is of course critical because, if the two gates are open simultaneously, energy stored in the sodium gradient will be lost. After sodium and the neurotransmitter have been released to the inside of the cell, the internal gate closes (step 3), and this closure is coupled to the opening of the external gate (step 4). Then, the transport cycle is completed. These last two steps are often referred to as the "return of the unloaded (or empty) transporter". In some transporters, such as the serotonin transporter SERT of the SLC6 family and the glutamate transporters of the SLC1 family, this return step is very slow. In these cases, potassium binds to the empty transporter, resulting in a relatively fast return of the potassium-loaded transporter. Upon release of potassium to the extracellular side, a new cycle can commence. Effectively, SERT and the glutamate transporters translocate potassium in the direction opposite that of sodium and the neurotransmitter. If the "return of the unloaded transporter" is selectively impaired by mutation, the full transport cycle cannot be executed. However, in such a case (where steps 1 and 2 are operative), reversible sodium and substrate translocation, measurable as sodium-dependent exchange of external radioactively labeled substrate with internal "cold" substrate, can still take place. Thus, in glutamate transporters, which require countertransport of potassium, selective lesion of the potassium-dependent reorientation by mutation leaves exchange intact.[26,27] Transport by the SLC6 neurotransmitter transporters but not by the glutamate transporters is also dependent upon chloride. In fact, the chloride ion is cotransported together with the neurotransmitter and the sodium ions.[28,29]

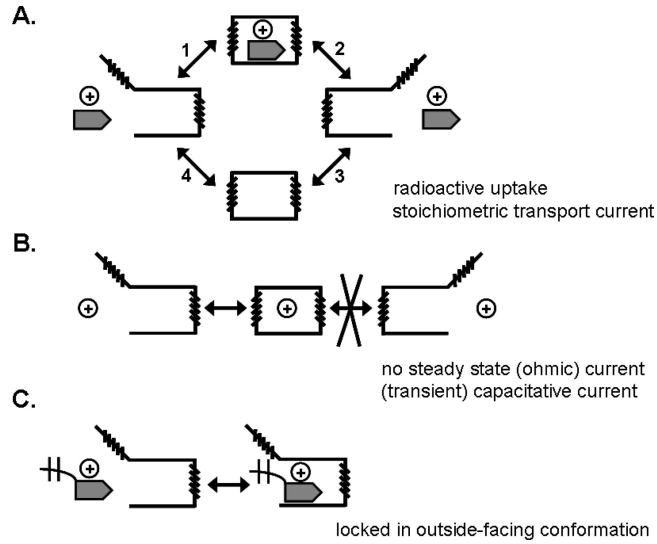

Figure 2. Schematic depiction of the transport cycle and other aspects of the action of neurotransmitter transporters. (A) Complete transport cycle. The empty transporter with the binding pocket open to the outside (left) binds the substrate and sodium ions (step 1) and proceeds through an occluded state where the substrate and cotransported ions are bound but inaccessible from either side of the membrane after which the internal gate opens (step 2), and the substrate together with the sodium ions are released to the intracellular medium. The empty inward-facing transporter then reorients by closing the internal gate (step 3) and opening the external one (step 4) to allow for a new transport cycle. (B) In the absence of the substrate, the closure of the external gate cannot be coupled to the opening of the internal gate. (C) Transporter where a nontransportable analogue of the substrate and sodium are bound cannot proceed through the occluded state (step 2 in A) and complete a transport cycle, and thus, the transporter remains locked in a state where only the external but not internal gate is open.

The transport process can be measured using uptake of radioactively labeled neurotransmitter as an assay, but because most transporters translocate excess positive charge in their transport cycle, the process can also be monitored as a sodium- and neurotransmitter- dependent steady-state current (steps 1–4 in Figure 2A).

Sodium can also bind to the transporters in the absence of the transported substrate. When oocytes, expressing the transporters, are perfused in a sodium-containing medium and are subjected to a voltage jump (interior negative), transient currents are observed. In contrast to the coupled currents, which are resistive, these transient currents are capacitative and are thought to reflect a charge-moving conformational change of the transporters following sodium binding.[8,29–31] When the membrane voltage is jumped back to the original holding potential, sodium is "pushed-off" the transporter back into the extracellular medium and a transient current in the opposite direction is observed. Although alternative explanations cannot be ruled out, a likely interpretation of the capacitative (transient) currents in terms of the "lock model" is that, upon sodium binding, the external gate closes. However, because the "driven" substrate is absent, the closure of the external gate cannot be coupled to the opening of the internal gate and the cation(s) is occluded in the binding site, having effectively moved through part of the membrane electric field (Figure 2B). In some transporters, the internal gate can briefly open even in the absence of substrate. This results in steady-state leak currents, carried by sodium or other ions (see for example refs 32 and 33). Sometimes the ions leaking through the transporter are different from those involved in coupled transport, although the substrates may modulate the leak pathway. This has been observed in the glutamate transporters.[34,35] It has been proposed that this process has a physiological role, namely, to clamp the membrane potential at a level enabling efficient glutamate uptake.[35–37]

If a nontransportable substrate analogue (blocker) is present together with sodium, this blocker will bind to the substrate-binding site. However, because the blocker is more bulky than the substrate, the external gate cannot close and this also prevents the opening of the internal gate (Figure 2C). Thus, the transporter is effectively locked in an outward-facing conformation. Therefore, if the cells are suddenly hyperpolarized in the presence of blocker, the sodium-dependent charge-moving conformational change cannot take place anymore and the transient currents are abolished.

2. SLC6/NSS Transporters

2.1. Overview of the Structure of the Bacterial SLC6 Homologue LeuT

The structure of a homologue of the SLC6/NSS transporters from the bacterium *Aquifex aeolicus* was published in a landmark paper by Yamashita et al.[14] Because a bound leucine molecule was observed in the binding pocket, the transporter was termed LeuT. Indeed, LeuT reconstituted into liposomes exhibits leucine transport, which is sodium-dependent.[14] Because the transporter also transports alanine,[38] it remains to be clarified what the natural substrate of LeuT is. The resolution of the LeuT structure is exceptionally high for a membrane protein (1.65 Å) and is extremely informative. Even though the transporter crystallized as a dimer, the functional unit is the monomer. The transporter monomer has 12 transmembrane domains, in accordance with predictions based on the deduced protein sequence of the GABA transporter GAT-1, the first cloned member of this family,[18] and with the topology of the serotonin transporter SERT, determined by experiment.[39] The structure of the monomer shows several features known from other transporter structures. One of these is an unanticipated internal structural repeat in the $LeuT_{Aa}$ monomer, such that TM1−TM5 and TM6−TM10 can be superimposed on each other by rotation around a pseudo-2-fold axis located in the plane of the membrane. The interface of these repeats forms the binding pocket of the transporter. Another feature is the unwinding of parts of the membrane-spanning domains, which was first observed in the calcium pump.[40] This enables several consecutive amino acid residues to participate in ion and/or substrate coordination, in contrast to every third or fourth residue in the case of an α helix. In LeuT, TM1 and TM6 are antiparallel to each other and have breaks in their helical structure approximately halfway across the membrane (Figure 3). These breaks expose main-chain carbonyl oxygen and nitrogen atoms for hydrogen bonding and ion binding. Residues on TM3, TM7, and TM8 also contribute to the binding of leucine and the two sodium ions, bound at the Na1 and Na2 sites. Some of these residues had already been implicated in ion and/or substrate binding by functional studies of mutants of several neurotransmitter transporters (cited in ref 14 and discussed in the next section). Therefore, it is evident that the structure reported by Yamashita et al.[14] is a physiologically relevant conformation of the transporter.

The structure reveals not only a completely new protein fold but also a crystal-clear view of the binding pocket, including the driven substrate and the two driving sodium ions (Figure 3). The sodium ions in the binding pocket are both close to the substrate, which is in direct contact, through the carboxyl group, with the sodium at Na1 (inset in Figure 3). Thus, it appears that, at least in this transporter and presumably in all of the amino-acid-transporting members of this protein family, the coupling between the "driving" ions and the "driven" substrate is as direct as can be. This direct coupling is an ingenious solution to minimizing leaks where the ion and/or the substrate might permeate through the transporter independently from each other. This mechanism has been proposed previously on the basis of indirect evidence from transporters of other families,[41,42] and it may turn out to be used by other transporters. Nevertheless, direct contact between sodium and the neurotransmitter may not occur in all transporters; for instance, not all of the substrates of transporters related to LeuT have carboxyl groups. In these cases, it seems that the carboxyl group is provided by a unique aspartate residue located on TM1.[14]

In the LeuT structure, the binding pocket is occluded; the external and internal gates are closed. Two ion pairs, one between the extracellular ends of TM1 and TM10 and the other between the intracellular ends of TM1 and TM8 contribute to these gates (Figure 3). The binding pocket of LeuT does not contain a chloride ion, and leucine transport is dependent upon sodium but not on chloride.[14] In other bacterial NSS transporters, such as the tryptophan transporter TnaT[43] and the tyrosine transporter Tyt1,[44] transport is also chloride-independent. In contrast, the eukaryotic NSS transport the neurotransmitter with sodium as well as with chloride.[29,45] As discussed in the Chloride-Binding Site section, the structural basis of this difference has been identified and it appears that the chloride-binding site is close to that of one of the sodium ions.[46,47]

On the basis of the LeuT structure, Yamashita et al.[14] suggested a putative mechanism based on the principle of alternating access (Figure 4). It was proposed that the extracellular and cytoplasmic segments, TM1b−TM6a and

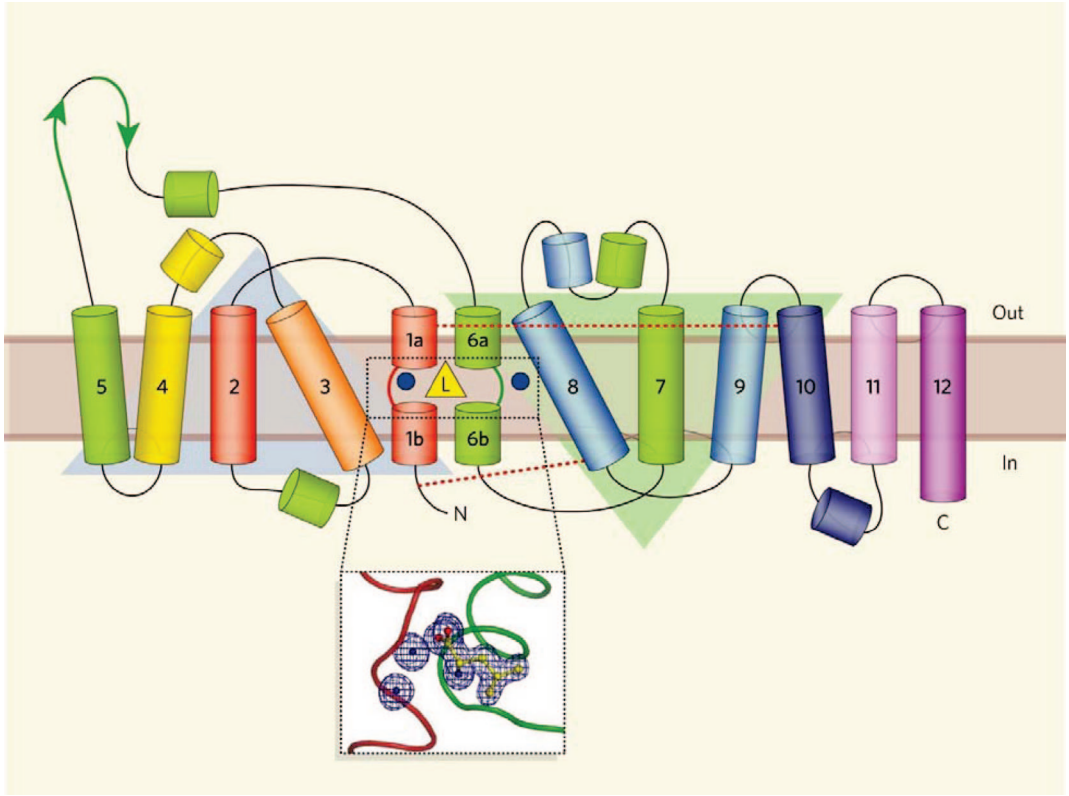

Figure 3. Topology of the leucine transporter. This figure shows $LeuT_{Aa}$ topology as determined by the crystal structure of the transporter obtained in the occluded conformation. Transmembrane domains (TMs) are numbered 1–12, and the oppositely oriented structural repeats encompassing TM1−TM5 and TM6−TM10 are shown as blue and green triangles. TM1 and TM6 are unwound halfway through the membrane, to form the binding pocket for the sodium ions (blue circles) and the leucine substrate (yellow triangle). The two dashed red lines connect the approximate positions of amino acids that interact as ion pairs to form parts of the external and internal gates, where the residues involved are spatially close to each other. The inset shows the binding pocket with the actual electron densities of leucine (carbon, yellow; oxygen, red; nitrogen, blue) and the two sodium ions (blue). Reprinted with permission from *Nature* (http://www.nature.com) ref 14. Copyright 2005 Nature Publishing Group.

TM1a−TM6b, respectively, may move relative to TM3 and TM8. This could result in the opening and closing of the extra- and intracellular gates (Figure 4). Future elucidation of structures of outward- and inward-facing conformations of LeuT or a related transporter will be required for more definitive conclusions on the transport mechanism.

2.2. Binding Pocket of the SLC6 Neurotransmitter Transporters

2.2.1. Binding of the Neurotransmitters

Even though LeuT crystallized as a dimer, each monomer had its own binding pocket, indicating that the monomer is the functional unit.[14] Also, because of the conservation of key residues of the LeuT-binding pocket throughout the entire SLC6 family, it is also likely that in the neurotransmitter transporters the monomer is the functional unit. Oligomerization has also been observed in the neurotransmitter transporters.[48–51] The role of the oligomer formation is not clear, but possibly this process is important during biosynthesis and delivery of the transporters to the plasma membrane.[49]

In the LeuT structure, the leucine molecule is coordinated by the sodium ion bound at the Na1 site, main-chain carbonyl oxygens from amino acid residues from TMs 1 and 6, main-chain amide nitrogens from TM6, and side-chain atoms of amino acid residues from TMs 3, 6, and 8[14] (Figure 5). Na1 interacts directly with the carboxy group of the bound leucine, and the only side chain interacting with this group is a hydroxyl from Tyr-108 of TM3.[14] This tyrosine is strictly conserved among all NSS family members and has been implicated in substrate binding and transport in GAT-1,[52] SERT,[53] and the glycine transporter GlyT2a.[54] In GAT-1, this is Tyr-140, and when this tyrosine is replaced by related residues, such as phenylalanine or tryptophane, transport activity is abolished. However, sodium can still bind to these mutants, as judged by their ability to perform the sodium-dependent transient currents.[52] However, unlike wild-type GAT-1, these transients cannot be suppressed by GABA or its nontransportable analogues, leading to the conclusion that tyrosine-140 is involved in the binding of GABA.[52]

While it is likely that the hydroxyl side chain of the conserved tyrosine coordinates the carboxy group of all of the NSS transporters of amino acids, this is clearly not the case for SERT, the dopamine transporter DAT, and the norepinephrine transporter NET. The substrates for these transporters are biogenic amines, which do not possess a carboxy group. Amino acid sequence alignment shows that a key difference is that, at TM1 position 24 of LeuT, all amino acid transporters have a glycine, whereas the biogenic amine transporters have an aspartate. It has been suggested earlier that the side-chain carboxy group of the conserved aspartate in the biogenic amine transporter fulfills the role played by the carboxy group of the substrate of the amino acid transporters of the family.[55] Consistently, in GAT-1, even small changes of the corresponding glycine residue impair GABA transport.[55] Indeed, when in the LeuT structure an aspartate is modeled instead of glycine-24 of LeuT, the

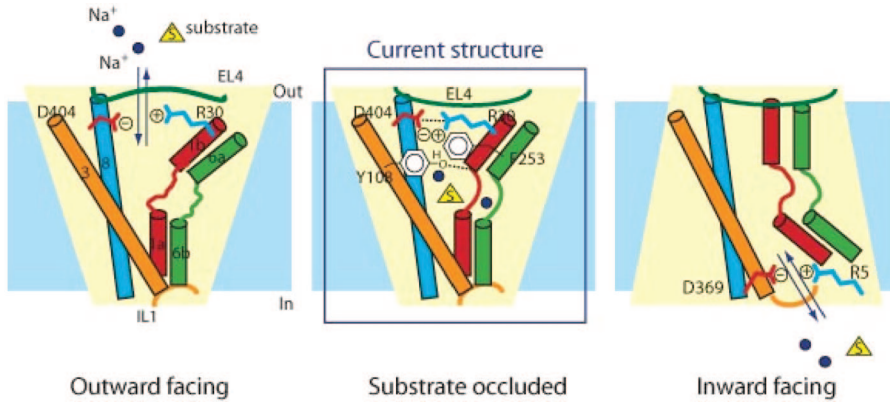

Figure 4. Schematic representation of possible conformational changes associated with the transport cycle. External access to the binding pocket is allowed if TM1b and TM6a are distant from the extracellular parts of TM3 and TM8, whereas internal access to the binding pocket is allowed if TM1a and TM6b are distant from the intracellular parts of TM3 and TM8. In the outward-facing conformation (left panel), only the external gate is open and the binding pocket is accessible only from the outside. In the substrate-occluded conformation (middle panel), which corresponds to the obtained crystal structure, the external gate is closed. In the inward-facing conformation (right panel), the internal gate opens and there is only cytoplasmic access to the binding pocket, and therefore, the substrate/sodium are released to the intracellular medium. Reprinted with permission from Reprinted with permission from *Nature* (http://www.nature.com) ref 14. Copyright 2005 Nature Publishing Group.

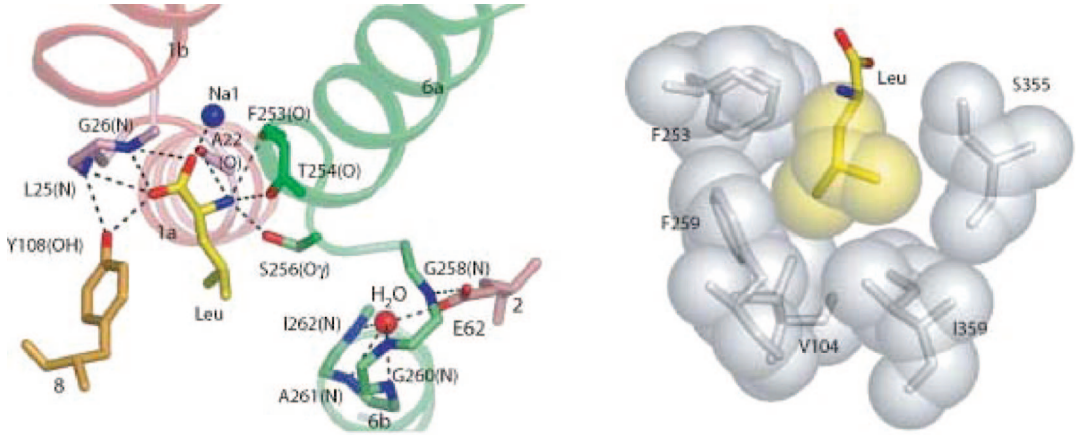

Figure 5. Leucine-binding site in $LeuT_{Aa}$. (Left panel) Hydrogen bonds and ionic interactions in the leucine-binding pocket depicted as dashed lines. (Right panel) hydrophobic interactions between the leucine molecule and the transporter. This figure shows van der Waals surfaces for the leucine side chain and interacting residues as spheres. Tyr-108 and Ser-256 are omitted from the figure for clarity. Reprinted with permission from *Nature* (http://www.nature.com) ref 14. Copyright 2005 Nature Publishing Group.

β-carboxy group of the aspartate can be positioned to within 1 Å of the amino group of the leucine substrate and within 3 Å of the sodium ion at Na1.[14] This indicates not only that in the biogenic amine transporters the carboxy group of the aspartyl residue may replace the carboxy group of amino acid substrates but also that this group coordinates a sodium ion (see below). By analogy with LeuT, this aspartyl carboxy group of the biogenic amine transporters is likely to form a hydrogen bond with the conserved TM3 tyrosine residue and perhaps also with the amine group of the substrate (see also ref 56), as suggested by mutagenesis studies with SERT.[57]

In LeuT, the only side chain interacting with the amino group of the leucine substrate is the hydroxyl of serine-256[14] (Figure 5). It is interesting to see that this serine is conserved in some NSS transporters of α-amino acids but not in the biogenic amine transporters (glycine replacement) nor in the four different GABA transporters (glycine replacement in GAT-1 and alanine replacement in the other three GABA transporters). If indeed the carboxy group of the unique aspartate of TM1 ligands the amine group of the biogenic amines, this serine would not be required. In the case of the GABA, the amino group is at the γ position and it is possible that the molecule could assume a "cyclic" conformation in the binding pocket of the GABA transporters, so that the γ-amino group of GABA can interact with its own carboxy group.

In the LeuT structure, the aliphatic side chain of the bound leucine resides in a hydrophobic pocket surrounded by side chains of amino acid residues from TM3 (Val-104 and Tyr-108), TM6 (Phe-253, Ser-256, and Phe-259), and TM8 (Ser-355 and Ile-359)[14] (Figure 5). The nature of these side chains probably determines the substrate specificity in the mammalian homologues. Indeed, in the transporters GlyT1 and GlyT2, which transport the small glycine molecule, residues at positions equivalent to those surrounding the isopropyl moiety of leucine in LeuT are replaced with amino acid residues of a larger size or a different shape. Missense, nonsense, and frameshift mutations in the gene encoding the human GlyT2 can cause the neurological disorder hyperekplexia.[58] One of these disease-causing mutations is the change of Trp-482, corresponding to Phe-259 of LeuT (Figure 5), to arginine. In agreement with the role of Phe-259 in the interaction with the side chain of the bound leucine in LeuT, the W482R mutation in GlyT2 causes defective interaction of the transporter with glycine. Importantly, this is a specific perturbation because sodium binding remains intact.[58] Moreover, the ability of GlyT1 but

not GlyT2 to interact with *N*-methyl glycine (sarcosine) can be traced down to a difference of a single amino acid corresponding to Ser-256 of LeuT (Figure 5). The smaller residue present in GlyT1 (glycine instead of serine) apparently allows the bulkier sarcosine to be accommodated in its binding pocket.[59]

The value of LeuT as a model for the NSS neurotransmitter transporters is further supported by the ability to convert the creatine transporter CRT to a GABA transporter, albeit slow and of low apparent affinity.[60] In contrast with the glycine transporters, the contact residues in SERT seem to be smaller than their LeuT counterparts [glycines instead of Ser-256 and Ile-359 (Figure 5)], apparently to accommodate the larger serotonin molecule. Attempts have been made to convert the tryptophan transporter TnaT and the tyrosine transporter Tyt1 to biogenic amine transporters by introducing the aspartate residue, unique for the biogenic amine transporters, at the position corresponding to Gly-24 of LeuT.[43,44] These attempts were unsuccessful, but it should be noted that at the important positions corresponding to Ser-256 and Ile-359 of LeuT (Figure 5), TnaT and Tyt1 have larger residues than the two glycines in the biogenic amine transporters. It is therefore possible that the positioning of the aromatic side chains in the binding pocket of the two amino acid transporters is different from that in their biogenic amine counterparts. Thus, additional mutations are required to convert an amino acid transporter into a biogenic amine transporter.

2.2.2. Sodium-Binding Sites

Two sodium ion binding sites, Na1 and Na2, were identified in the LeuT structure.[14] Na1 is coordinated by the carboxy oxygen of the bound leucine, two main-chain carbonyl oxygens of Ala-22 (TM1) and Thr-254 (TM6), and three side-chain oxygens from Asn-27 (TM1), Asn-286 (TM7), and Thr-254 (TM6)[14] (Figure 6A). The conservation of the latter three residues in the NSS family is very high; Asn-27 is fully conserved, whereas Asn-286 is conserved in most family members and, in some others, it is replaced by Asp, which also has an oxygen-containing side chain. Thr-254 is replaced in most NSS transporters by the related Ser. This conservation points to the existence of a similar Na1 site throughout the family. Na2 is coordinated by three main-chain carbonyl oxygens, from Gly-20 and Val-23 (TM1) and from Ala-351 (TM8), as well as by two side-chain hydroxyl oxygens from Thr-354 and Ser-355 (Figure 6B). The conservation of Thr-354 is less than that of residues coordinating Na1 (Ser, Gly, or Asp replacements), but Ser-355 is almost fully conserved throughout, with conservative substitutions (to Thr) only in the two glycine transporters. The conservation of the residues coordinating Na1 and Na2 suggest that these same residues may be involved in the coordination of sodium in the neurotransmitter transporters of this family. Further support for this idea was obtained using functional analysis of mutations in the GAT-1 counterparts of these residues.[61]

GAT-1 catalyzes electrogenic sodium/chloride/GABA cotransport with a stoichiometry of 2:1:1.[8,45,62,63] Even though lithium by itself does not support GABA transport,[64,65] it has been proposed that lithium can replace sodium at one of the binding sites but not at the other.[65] Four of the five GAT-1 residues, corresponding to those whose side chains participate in the two sodium-binding sites of LeuT, are conserved. Only aspartate-395 from TM8 replaces the Na2 residue threonine-354 of LeuT. At varying extracellular sodium concentrations,

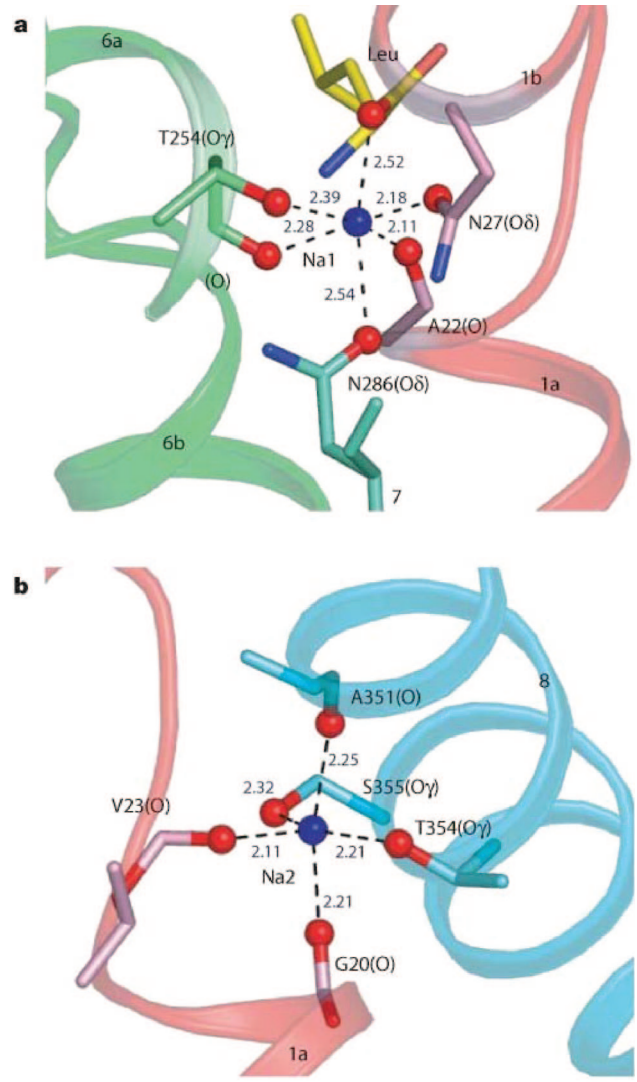

Figure 6. Sodium ion binding sites in $LeuT_{Aa}$. (A) Sodium ion at the Na1 site is liganded by amino acid residues from TM1a, TM1b, TM6a, and TM7 and the carboxy group of the leucine substrate. (B) Sodium ion at the Na2 site is liganded by residues from TM1a and TM8. Distances (in angstroms) are shown in blue letters. Reprinted with permission from *Nature* (http://www.nature.com) ref 14. Copyright 2005 Nature Publishing Group.

lithium stimulates sodium-dependent transport currents as well as [^{3}H]-GABA uptake in wild-type GAT-1, and the extent of this stimulation is dependent upon the GABA concentration.[61] In mutants where aspartate-395 is replaced by threonine or serine, the stimulation of transport by lithium is abolished.[61] Moreover, these mutants are unable to mediate the lithium leak currents. Even though their transport properties are severely impacted, this phenotype is not observed in mutants at the four other positions. Thus, at saturating GABA, the site corresponding to Na2 behaves as a low-affinity sodium-binding site, where lithium can replace sodium. Probably GABA participates in the other sodium-binding site, just like leucine does in the Na1 site, and this site determines the apparent sodium affinity of transport at limiting GABA concentrations.[61]

Even though it appears that, also regarding sodium coordination, the LeuT structure is a good model for the SLC6 neurotransmitter transporters, several of these transporters have a different sodium/substrate stoichiometry. For instance, whereas this stoichiometry of GlyT1 is also 2:1, in the case of GlyT2 it is 3:1.[29] The residues coordinating

Na1 in LeuT (Figure 6A) are conserved in both glycine transporters (with Ser instead of Thr-254), indicating that this site is used by both transporters. The GlyT2 residues Asp-577 and Thr-578 correspond to Thr-354 and Ser-355, which coordinate Na2 in LeuT (Figure 6B) and to Asp-395 and Ser-396 of GAT-1. Thus, GlyT2 apparently also uses a site similar to Na2, but it is not clear where the third sodium-binding site is. GlyT1 probably does not use the Na2 site, because it lacks an oxygen-containing side chain at the position corresponding to Thr-354 of LeuT. It is therefore possible that the third as yet unknown Na-binding site is also used by GlyT1. SERT has the same five oxygen contributing side-chain residues at Na1 and Na2 as GAT-1, but only one sodium appears to be cotransported with serotonin.[66,67] It is possible that SERT binds two sodium ions, but upon reorientation of the binding sites, only one gets released to the cytoplasm and the other remains bound to the transporter. It is of interest to note that in the invertebrate homologue KAAT, which can use either Na^+ or K^+ to drive the uptake of neutral amino acids, the residue corresponding to Asn-286 of the Na1 site of LeuT is Asp. Mutation of the Asp to Glu abolishes the ability of K^+ but not Na^+ to drive transport. It will be interesting to determine the stoichiometry of wild-type and mutant KAAT, because the Na2 site may not be functional in this transporter (Gly and Ser instead of Thr-354 and Ser-355 of LeuT).

2.2.3. Chloride-Binding Site

In several eukaryotic NSS, it has been shown that chloride is cotransported with the neurotransmitter and sodium.[29,45,68,69] In contrast, transport by the bacterial NSS transporters LeuT, Tyt1, and TnaT is chloride-independent,[14,43,44] and consistently, the only Cl^- ion identified in the LeuT structure interacts with nonconserved residues in external loops at a site far away from the binding pocket.[14] Very recently, the genuine chloride-binding site has been identified by two groups each using a different approach.[46,47]

In one of the approaches, buried regions in the LeuT structure, capable of accommodating a negative charge, were identified. This was performed by calculating the pK_A of the ionizable residues in the protein, and this led to the identification of Glu-290 located in TM7 of LeuT.[46] Also in TnaT, a negatively charged residue (Asp) occupies this position, whereas in Tyt1, a negatively charged residue is only one helical turn away on TM7. Strikingly, a serine occupies this position in all neurotransmitter transporters of the family, leading to the idea that the fixed negative charge present in the bacterial transporters may be replaced by chloride and that the conserved serine could be involved in the liganding of this anion. Indeed, introduction of a Glu or Asp at this position in SERT gives rise to chloride-independent transport, even though the level of transport is very low and does not exceed the chloride-independent transport by wild-type SERT.[46] However, as explained below, an at least partial explanation of this low activity has been provided by the study of chloride-independent GAT-1 mutants.[47]

The other approach to identify the chloride-binding site was based on the crystal structure of a chloride/proton antiporter.[70] In this structure, the chloride ions are coordinated by main-chain NH groups and side-chain hydroxyls from serine and tyrosine residues.[71,72] Analysis of mutants of conserved hydroxyl-containing residues located in the transmembrane domains of GAT-1 showed that mutation of Ser-331, which is equivalent to Glu-290 of LeuT, to negatively charged residues led to chloride-independent transport.[47] Similar results were also obtained with the GABA transporter GAT-4 and the dopamine transporter DAT.[47] Significantly, the reciprocal mutants in LeuT and Tyt1 rendered these transporters dependent upon chloride.[47] Moreover, introduction of residues with a smaller side chain than Ser at position 331 of GAT-1, namely, Ala and Gly, potentiated the ability of anions larger than chloride to stimulate transport, as if the smaller side chain created more space at the chloride site.[47] The rate of chloride-independent GABA transport by S331E is low. However, it is nevertheless 5–10-fold that of the chloride-independent transport by wild-type GAT-1.[47] An explanation of the relatively low rate of transport by S331E is that, in contrast to the wild type, where the chloride is released to the cytoplasm (Figure 7A), the fixed negative charge of the introduced glutamate residue remains on the S331E transporter (Figure 7B). This could potentially limit the reorientation step of the empty transporter and slow transport. Indeed, lowering the internal pH in reconstituted S331E transporters, which is expected to neutralize the negative charge (Figure 7B), increases the rate of transport of S331E but not of the wild type, by an order of magnitude.[47] Such a stimulation was not observed when exchange, which does not involve the return of the "empty" transporter (parts A and B of Figure 7), was monitored,[47] indicating that either a mobile (chloride) or a fixed negative charge is required during the sodium-coupled substrate translocation step.

The proximity of the putative chloride and Na1 sites (parts C and D of Figure 7)[46,47] and the requirement for a mere negative charge, during the translocation of GABA and the two sodium ions but not during the return step,[47] suggests that the role of chloride is mainly to compensate the multiple positive charges. This proximity also provides a satisfying explanation for the ability of chloride to increase the apparent affinity of GAT-1 for sodium.[9] An important experimental prediction emerging from these studies is that transport by the bacterial homologues may involve countertransport with protons (see parts A and B of Figure 7).

2.3. NSS Transporter "Gates" and Conformational Changes

The LeuT structure represents a conformation of the transporter where leucine and two sodium ions are occluded in the binding pocket[14] (Figure 3), and this is conceptually equivalent to a situation where the extra- and intracellular gates are both closed (top of Figure 2A). In the case of the extracellular gate of LeuT, only a few residues obstruct the leucine- and sodium-binding sites.[14] Closest to the substrate are the two aromatic residues, which make direct contact with the leucine molecule. These are the absolutely conserved Tyr-108 (TM3) and the highly conserved Phe-253 (except for a few cases where this residue is conservatively replaced by Tyr) of TM6, which have been discussed in the Binding of the Neurotransmitters section. Just "above" this pair is a conserved pair composed of Arg-30 (TM1) and Asp-404 (TM10), which interact indirectly via a pair of water molecules in the LeuT structure.[14] Arg-30 also interacts with Phe-253 and the fully conserved Gln-250 (TM6). The equivalent residues of Arg-30 and Gln-250 in GAT-1 (Arg-69 and Gln-291, respectively) are absolutely essential for GABA transport,[55,73,74] and this together with the strong conservation of the five above-mentioned residues strongly suggest that the neurotransmitter transporters have an external

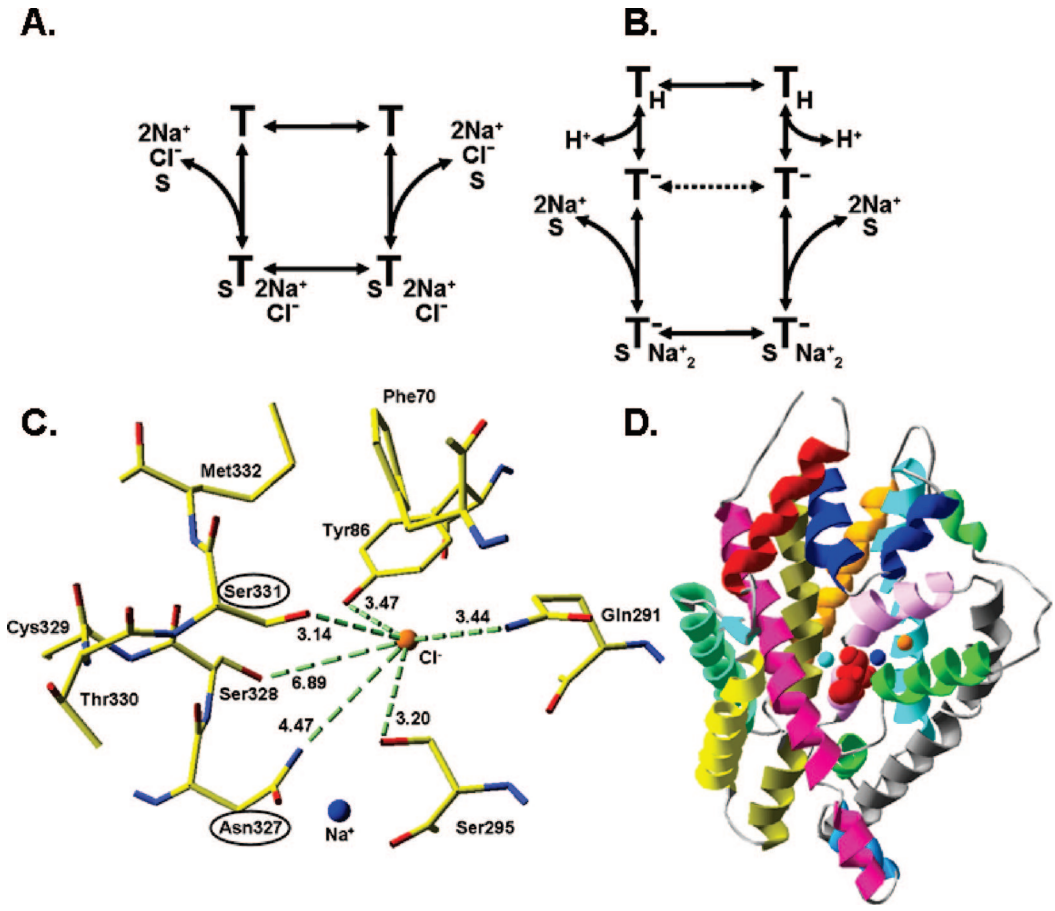

Figure 7. Transport cycle in chloride-dependent and -independent members of the SLC6. (A) Transport cycle in a chloride-dependent NSS, such as wild-type GAT-1. The transporter (T) binds two sodium ions, one chloride ion, and the substrate (S) from the outside of the cell (left) and translocates and releases the substrate and cotransported ions to the inside (right). Afterward, the empty inward-facing transporter reorients to face the outside again. (B) In chloride-dependent members of the NSS (or in the case of the GAT-1 S331E mutant), the constant negative charge on the transporter (T^-) enables the translocation of sodium and the substrate even in the absence of chloride, but the return step of the empty transporter is slow (dashed, double-headed arrow) unless accelerated by neutralization of the negative charge, such as by protonation (TH). (C and D) Schematic illustration of the putative chloride-binding site in eukaryotic NSS. The chloride ion (orange), the sodium ions at Na1 (blue) and Na2 (cyan), and the bound leucine (red, space-filling representation) are shown in the context of the overall LeuT structure. (C) Glu 290 of LeuT was replaced with serine (equivalent to Ser 331 of GAT-1); a chloride ion was introduced at the site of the γ-carboxyl group of the original glutamate residue; and residues within 5 Å of the introduced serine were replaced by their GAT-1 counterparts. Numbering refers to GAT-1, and distances are given in angstroms. TM10–TM12 and Asn 66 (roughly positioned "in front of" Asn 327) have been removed for clarity. Parts C and D were prepared using a Deep-View Swiss-PDB viewer, downloaded from the ExPaSy Proteomics Server.

gate similar to that of LeuT. Interestingly, although completely inactive in transport, GAT-1-R69K still exhibits the sodium-dependent transient currents.[55] A possible explanation is that, with a lysine present at this position, the external gate still can close but the mutation interferes with the coupling of the closure of the extracellular gate and the opening of its intracellular counterpart (see also Figure 2).

The intracellular gate of LeuT is much more substantial than its extracellular counterpart. Among the residues participating in the formation of the intracellular gate of LeuT are Arg-5 (TM1) and Asp-369 (TM8), which form a salt bridge, and Trp-8 (TM1), Tyr-265, Ser-267, and Tyr-268 (TM6).[14] Except for Tyr-265, the conservation at the other four positions is extremely high, and mutations in the GAT-1 counterparts of Arg-5 and Trp-8 and the DAT counterpart of Tyr-268 disrupt transport.[64,75] This indicates that in the neurotransmitter transporters also the internal gate is very similar to that of LeuT. GAT-1 mutants at Arg-44 and Trp-47, corresponding to Arg-5 and Trp-8 of LeuT, were defective in net GABA transport but capable of sodium-dependent exchange of [^{3}H]-GABA with unlabeled GABA[64] (reversible steps 1 and 2 in Figure 2). This suggests that the perturbations caused by the mutations at the intracellular gate perturb the coupling between the two gates when the transporter is empty but not when it is loaded (Figure 2). Very recently, two groups determined the structure of LeuT in complex with substrate and tricyclic antidepressant (TCA).[76,77] Although it is not likely that this new structure is a good model of competitive TCA binding to the biogenic amine transporters (see ref 78 for a discussion), the new structures have some interesting implications that satisfactorily explain the noncompetitive binding of the TCAs to LeuT.[76,77] TCAs bind in an extracellular-facing vestibule about 11 Å above the substrate and the two sodium ions, just extracellular of the conserved Arg-30/Asp-404 pair, stabilizing the extracellular gate in a closed conformation. The overall structure of the LeuT structures stabilized by TCAs is similar to that of the original LeuT structure, with two exceptions: whereas in the original structure Arg-30 and Asp-404 interact indirectly via a pair of water molecules, in the TCA-containing structure, the side chains of the two residues form a direct salt bridge and the intervening water

molecules are displaced.[76,77] The extracellular vestibule is also lined by the hairpin of extracellular loop 4, and in the TCA-containing structure, the tip of this loop is flipped toward the extracellular side of the transporter. Interestingly, in GAT-1 and SERT, this extracellular loop undergoes accessibility changes during transport.[79,80]

Several of the NSS neurotransmitter transporters exhibit substrate-induced currents, which are larger than expected from their transport stoichiometry, and this is especially true for the biogenic amine transporters (see for instance refs 32 and 33). In terms of the lock model (Figure 2), this can be interpreted as if sometimes both gates are open. Such uncoupled currents have also been seen to some extent in GAT-1,[69] whereas they apparently do not occur at all in the glycine transporters.[29] Perhaps uncoupling can be easier tolerated in the case of the biogenic amine transporters, which, because of the metabolism of the intracellular amines by monoamine oxidase, have to cope with smaller gradients of their substrates than the other transporters. Sometimes the leak currents can be ascribed to a particular conformation of the transporter. For instance, in GAT-1 in the absence of sodium and in the presence of lithium, a leak mode is observed,[9] which represents a distinct conformation of the transporter.[81] In the presence of low concentrations of sodium, the lithium-leak currents are inhibited, apparently because sodium mediates the transition from the leak mode to the coupled mode of the transporter.[55,65] Mutation of Asp-395 at the Na2 site of GAT-1 to uncharged residues abolishes not only the ability of lithium to stimulate GABA transport but also the lithium-leak currents.[61] This indicates that also in the leak mode the Na2 site is used for ion permeation. There is also biochemical evidence for conformational transitions of the transporters. In GAT-1, GABA can protect against the proteolytic cleavage of the transporter, provided that the two cosubstrates, sodium and chloride, are also present.[82]

The accessibility of engineered cysteines at many positions of the neurotransmitter transporters has been inferred from the functional impact on the activity of these cysteine mutants by sulfhydryl reagents. Often this reactivity is influenced by the presence of sodium, the neurotransmitter, and/or non-transportable substrate analogues. In several cases, these positions are far away from the substrate-binding site, as defined by the LeuT structure.[14]Therefore, it is unlikely that these residues are directly occluded by substrate or ion binding, but the changes in reactivity are likely to reflect conformational changes in response to the occupation of the binding pocket. Such accessibility changes have been observed with cysteines introduced in many structural elements of the transporters, including the already mentioned extracellular loop 4,[79,80] TM1,[83,84] TM3,[85] and TM8.[86] Of particular interest is the observation that accessibility of a cysteine residue at one of the TM3 positions of the norepinephrine transporter NET was increased by the nontransportable analogue cocaine, which is expected to lock the transporter in its outward-facing conformation. On the other hand, the external accessibility at this position was decreased by the transportable substrate dopamine. This suggests that the engineered cysteine becomes either occluded or accessible to the inside of the cell during transport.[85] Increased accessibility of cysteines introduced in the cytoplasmic half of TM5 of SERT to a membrane-permeant sulfhydryl reagent was observed in the presence of serotonin, whereas cocaine decreased it.[87] These observations indicate that when the transporter becomes inward-facing, the cytoplasmic half of TM5, which is occluded in the LeuT structure,[14] lines an aqueous pathway, leading from the binding pocket to the cytoplasm.[87]

The ability to undergo multiple conformational changes requires the presence of elements that permit flexibility of the structure of the transporter. Glycines can introduce considerable flexibility to proteins, and recently, a glycine residue has been implicated to act as a hinge in potassium channels.[88] Moreover, evidence has been presented suggesting that glycine residues, engineered into the proton-coupled lactose transporter, confer conformational flexibility to it.[89] A conserved glycine residue at the top of TM2 of GAT-1 has been identified, which may play a role in the conformational changes during transport.[90] Replacement of glycine-80 by cysteine results in the complete loss of [^{3}H]-GABA uptake, but oocytes expressing this mutant exhibit the sodium-dependent transient currents. When sodium is removed and subsequently added back, the transients by G80C do not recover, as opposed to the wild type, where recovery is almost complete. Remarkably, the transients by G80C can be restored after exposure of the oocytes to either GABA or a depolarizing prepulse. These treatments also result in a full recovery of the transients by the wild type. Whereas, in the wild type, lithium-leak currents are observed after prior sodium depletion, this is not the case for the glycine-80 mutants, again, unless GABA is added or the oocytes are subjected to a depolarizing prepulse. Thus, glycine-80 appears to be essential for conformational transitions in GAT-1. When this residue is mutated, removal of sodium results in "freezing" the transporter in one conformation from which it can only exit by compensatory changes induced by GABA or depolarization. These results can be explained by a model invoking two outward-facing states of the empty transporter and a defective transition between these states in the glycine-80 mutants.[90]

3. Glutamate (SLC1) Transporters

3.1. Properties of SLC1 Neurotransmitter Transporters

The five known glutamate transporters from the brain and the retina have an overall amino acid sequence identity of around 50% and include the glial transporter glutamate transporter-1 (GLT-1)[6] and the neuronal excitatory amino acid carrier (EAAC1).[23]Glutamate uptake is an electrogenic process,[91,92] in which the transmitter is cotransported with three sodium ions and one proton,[93,94] followed by the countertransport of one potassium ion.[26,95,96] In the recent models of the transport cycle, at least one sodium ion binds before the acidic amino acid substrate, and subsequent substrate binding enables the binding of the additional sodium ions.[97,98] The mechanism, which involves separate half-cycles of sodium-coupled glutamate and potassium translocation, respectively (Figure 8), is supported by the fact that mutants impaired in potassium interaction are locked in an obligatory exchange mode.[26,27] Glutamate transporters mediate two distinct types of substrate-induced sodium-dependent steady-state current: an inward-rectifying current, because of the transmembrane movement of two positive charges for each transported glutamate molecule (electrogenic transport current), and an "uncoupled" current, carried by chloride ions and further activated by substrates of the transporter.[34,35,99] Moreover, when the membrane voltage is suddenly changed in the absence of substrate, sodium-dependent transient currents are observed. These transients presumably represent a charge-moving conformational change following

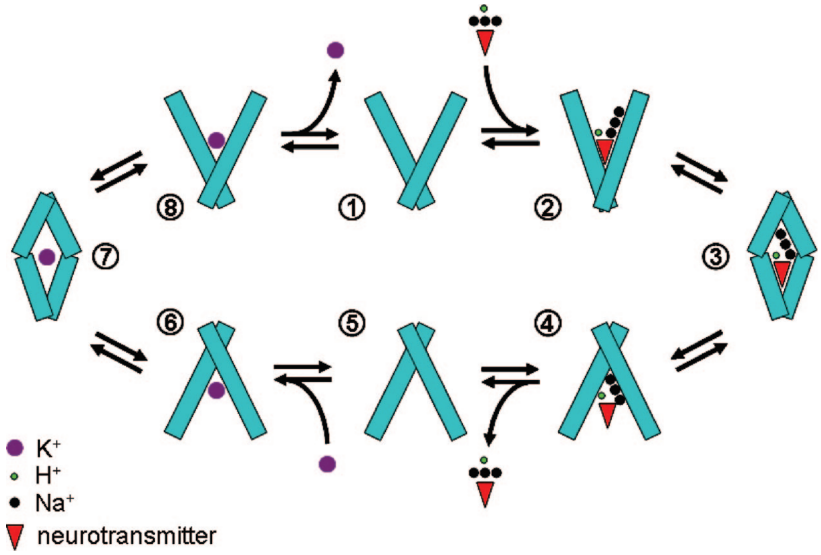

Figure 8. Transport cycle of glutamate. The empty-outward facing transporter (1) binds the substrate (red triangle) and cosubstrates ($3Na^+$ and $1H^+$, black and green circles, respectively) (2) and moves through the occluded state, where the substrate/cosubstrates are inaccessible from either side of the membrane (3), before the internal gate opens (4), and the substrate/cosubstrates are released to the intracellular compartment (5). The empty-inward facing transporter then binds internal potassium (6), and both gates close (7) before the transporter reorients to face the external side (8), where the bound potassium is released. The scheme depicts the two half cycles of glutamate transport; the sodium-coupled glutamate translocation (steps 2−4) and the potassium efflux (steps 6−8).

binding/debinding of sodium.[31] The addition of a transportable substrate enables electrogenic transport and thereby converts the transient current into a steady-state current.[31] On the other hand, nontransportable analogues block the transient currents, presumably by locking the transporter in an outward-facing conformation.[31] These observations on the presteady-state currents are similar to those on NSS and other ion-coupled transporters, and their explanation is the same as illustrated in Figure 2.

3.2. Overview of the Structure of the Archeal SLC1 Homologue Glt_{Ph}

No functional information on the archeal homologue, named Glt_{Ph}, was available when its first structure was reported.[13] However, it was clear that Glt_{Ph} belonged to the SLC1 family of glutamate and other transporters not only because of sequence conservation but also because the features revealed in the structure were in excellent agreement with functional studies on eukaryotic glutamate transporter mutants (see the next section) and also with the experimentally determined membrane topology of the glutamate transporter GLT-1 from brain and its counterpart GltT from *Bacillus stearothermophilus*.[100–102] In a subsequent study, it was shown that Glt_{Ph} is an aspartate transporter.[15] The Glt_{Ph} structure has a relatively low resolution as compared to that of LeuT,[14] and therefore, our knowledge on the mechanism of transport by SLC1 transporters is less detailed than that by their SLC6 counterparts. The Glt_{Ph} structure revealed a bowl-shaped structure, formed by a trimer of the transporter, with a solvent-filled extracellular basin extending halfway across the membrane bilayer.[13] At the bottom of the basin three independent binding sites were observed, one in each transporter monomer, suggesting that the monomer is the functional unit. Support for the idea that each monomer functions independently comes from studies with the bacterial glutamate transporter GltT[103] and the neuronal glutamate transporter EAAC1/EAAT3.[104–107] Each of the substrate-binding sites is cradled by two re-entrant loops or helical hairpins, HP1 and HP2, reaching from opposite sides of the membrane (parts A and B of Figure 9). The tips of these two hairpins come into very close proximity.[13] This is in beautiful agreement with earlier studies, indicating that cysteines, introduced at each of the two corresponding re-entrant loops of the rat brain astroglial glutamate transporter GLT-1, could be cross-linked within the monomer.[108] A nonprotein electron density, apparently corresponding to the bound substrate, was found in the binding pocket in close proximity to conserved amino acid residues critical for function in the corresponding transporters from brain. This includes an arginine residue implicated in the binding of one of the two carboxyl groups of the substrate.[109] The location of this buried substrate site is reminiscent of the occluded leucine site in LeuT. Because of the limited resolution of the Glt_{Ph} structure, it was impossible to visualize bound sodium and to see if one of the two carboxy groups of the substrate directly interacts with the cations, as has been observed for LeuT.[14] To get some ideas on the location of the sodium sites, the robust anomalous scatterer thallium has been used as a sodium surrogate.[15] As will be discussed in the next section, it is not clear yet if the observed thallium sites faithfully represent the physiological sodium sites.

On the basis of the initial Glt_{Ph} structure, it was proposed that glutamate transport is achieved by movements of the hairpins that allow for alternating access to either side of the membrane.[13] The first support for this idea was obtained when Gouaux and his collegues solved the crystal structure of Glt_{Ph} in complex with D,L-threo-β-benzyloxyaspartate (TBOA),[15] a nontransportable substrate analogue in the eukaryotic transporters[110] as well as in Glt_{Ph}.[15] The overall structure of the transporter was found to be similar in the aspartate- and TBOA-bound complexes, except that in the TBOA-bound structure HP2 adopts an "open" conformation. HP2 moves by around 10 Å from its position in the aspartate-bound complex toward the extracellular loop connecting TM helices 3 and 4. This loop itself also moves closer to HP2, enabling direct contacts. These movements expose the substrate-binding site to the extracellular solution, in agreement with the concept illustrated in Figure 2, and support

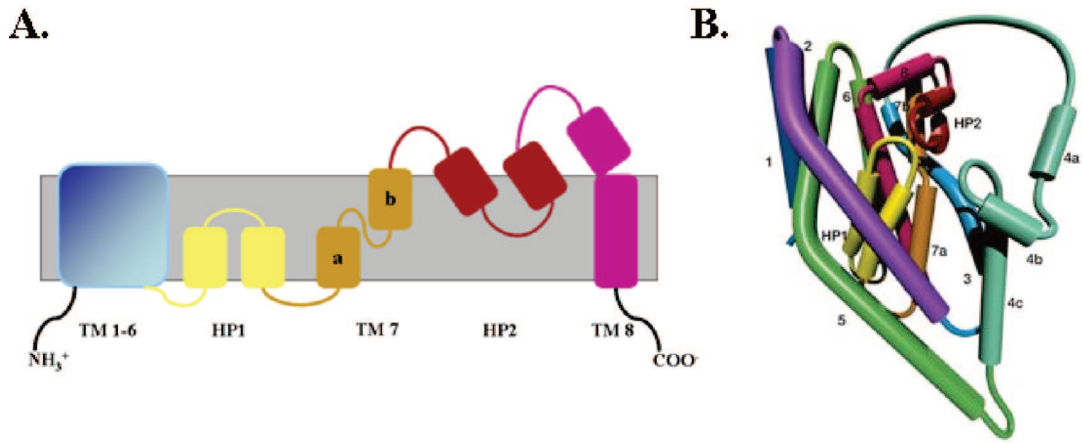

Figure 9. Topology and structure of the Glt_{Ph}. This figure shows membrane topology (A) and structure (B) of the bacterial glutamate protomer viewed in the plane of the membrane. Transmembrane (TM) helices (1−8) and hairpins (HP1 and HP2) are labeled. The same coloring pattern was adopted for all panels in Figures 9 and 10. B is reprinted with permission from *Nature* (http://www.nature.com) ref 13. Copyright 2004 Nature Publishing Group.

the hypothesis that HP2 represents the external gate of the transporter.[15]

3.3. Binding Pocket of the Eukaryotic Glutamate Transporters

As mentioned in the previous section, the features revealed in the Glt_{Ph} structure are in excellent agreement with functional studies on eukaryotic glutamate transporter mutants. Many of the amino acid residues of the eukaryotic transporters, which were inferred to be important for the interaction with glutamate and the co- and counter-transported ions, face the binding pocket and are close to the substrate[13,15] (Figure 10). These include (GLT-1 numbering) Ser-440 (apex of HP2)[42] and Thr-400 (TM7, corresponding to Thr-314 of Glt_{Ph}, Figure 10B),[111] which are determinants of sodium selectivity, Asp-474[112] and Arg-477[109] (TM8, corresponding to Asp-394 and Arg-397 of Glt_{Ph}, Figure 10B), important for the interaction with the amino and carboxyl groups of the substrate, respectively, and Tyr-403[27] (TM7, corresponding to Tyr-317 of Glt_{Ph}, Figure 10B), important for the interaction with potassium. Glu-404 is important for potassium interaction as well,[26] but this residue is not conserved in Glt_{Ph} (Gln-318, Figure 10B) and other bacterial glutamate transporters. It therefore appears that transport by the bacterial and archeal homologues does not involve the potassium-dependent relocation step (Figure 8). In the Glt_{Ph} structure, Gln-318 is actually pointing away from the binding pocket (Figure 10B). However, it is possible that, in the empty Glt_{Ph} transporter (and the potassium-bound eukaryotic glutamate transporters), the side chain at this position may point toward the binding pocket.

The Glt_{Ph} counterparts of two additional residues, important for the interaction of the eukaryotic transporters with sodium, point away from the binding pocket in the published structure; these are Asn-396 and Asp-398 of GLT-1 of the highly conserved NMDGT motif (unwound part of TM7, Figure 9). In the Glt_{Ph} structure, these residues form a β bridge.[13] Functional studies using a double mutant, where these residues were interchanged, indeed suggest that the interaction of these two residues is important during the glutamate translocation step, but the side chains of these residues themselves are required for the subsequent potassium relocation step.[113] One explanation could be that these residues participate in sodium and potassium binding to the

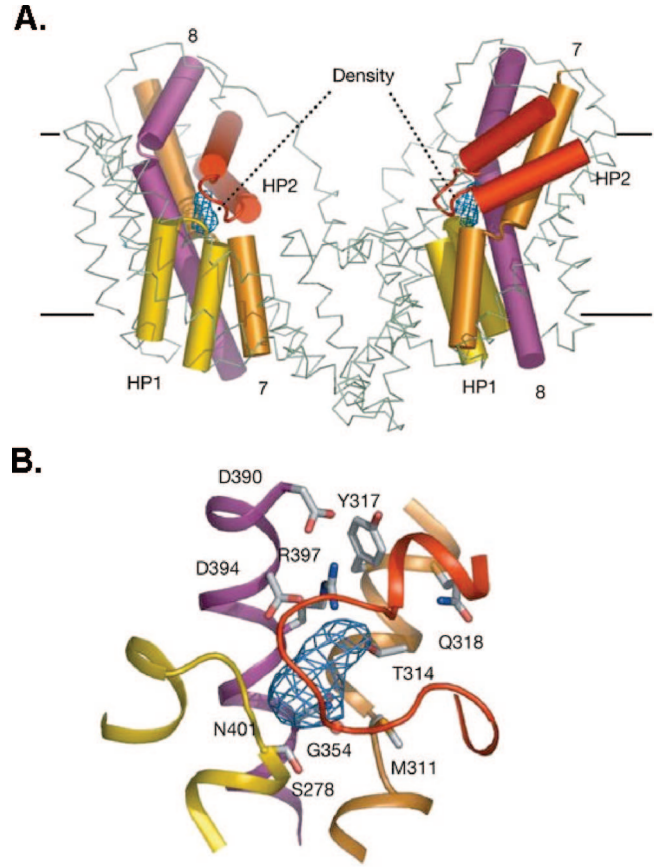

Figure 10. Substrate-binding site in Glt_{Ph}. Two monomers viewed in the membrane plane with N-terminal cylinders represented by an α-carbon trace and with HP1, TM7, HP2, and TM8 shown as cylinders. The substrate-binding site (blue mesh) and amino acid residues implicated in glutamate and ion binding (stick) are shown, both in the overall structure of the transporter (A) and in close-up view (B). Reprinted with permission from *Nature* (http://www.nature.com) ref 13. Copyright 2004 Nature Publishing Group.

glutamate-free transporter. The concept of overlapping binding sites for sodium and potassium has been suggested for the sodium pump.[114] Consistently, Asp-398 has been shown to be important for the binding of sodium to the glutamate-free transporter.[115] The location of the cation-binding sites is still an open issue, because the resolution of the Glt_{Ph} structure is not sufficient to observe sequestered

sodium ions. To circumvent this problem, the heavy metal cation Tl^+ was used as a sodium surrogate and two Tl^+-binding sites were identified.[15] However, it is questionable whether these Tl^+-binding sites are analogous to the Na^+-binding sites in the mammalian transporters and in Glt_{Ph}, because Tl^+ does not support substrate transport. Moreover, the properties of a mutant of the EAAC1 equivalent of the aspartate residue involved in Tl^+ binding[15] do not support its role in sodium binding.[115,116] Clearly, it is too early to decide if the intriguing observation that in the LeuT structure one of the two sodium ions makes direct contact with the substrate[14] also applies to the glutamate transporters. Consistent with such a scenario, recent evidence for a functional interaction of the driving ions (sodium or lithium) and the substrate has been obtained for the EAAC1 transporter.[117]

3.4. Glutamate Transporter "Gates" and Conformational Changes

The Glt_{Ph} structure represents a static picture of a substrate-occluded conformation of the transporter.[13] The TBOA-bound structure,[15] where the proposed extracellular gate, HP2, has moved toward the extracellular space, resembles the outward-facing conformation of the transporter. However, during a translocation cycle, the transporter transits through many other conformations. Accessibility studies can provide evidence for such conformational changes occurring during transport. It has been shown that the aqueous accessibility of cysteine residues, engineered into part of the re-entrant loop HP2 (HP2a), to membrane impermeant thiosulfonate (MTS) reagents is modulated by the presence of sodium, glutamate, and the nontransportable analogue dihydrokainate.[118] On the other hand, most of HP1 is not accessible to these impermeant reagents from the extracellular side,[102] in agreement with its location toward the intracellular side and its assignment as part of the intracellular gate of the transporter.[13] Opening of this intracellular gate, to allow for the dissociation of sodium and substrate to the cytoplasm, is predicted to increase its accessibility to cysteine-modifying reagents from the cytoplasm. Consistently, many of the cysteine residues engineered into HP1 become more accessible to the membrane permeant *N*-ethylmaleimide in the presence of external potassium,[119] a condition expected to increase the proportion of inward-facing transporters (Figure 8).

A beautiful example of accessibility changes compatible with alternating access has been observed with a cysteine engineered at the position Tyr-403 of GLT-1 (equivalent to Tyr-317 of Glt_{Ph}, Figure 10B), important for the interaction of the transporter with potassium.[27] The nontransportable analogue dihydrokainate, expected to increase the number of outward-facing transporters, increased the aqueous accessibility of the Y403C mutant, whereas transportable substrates decreased it.[120]

In addition to the ion-coupled glutamate translocation, glutamate transporters mediate a thermodynamically uncoupled chloride flux activated by two of the molecules that they transport, sodium and glutamate.[35,121] Coexpression studies of wild-type and mutant transporters indicate that not only the glutamate-binding sites and transport pathways but also the chloride channels reside in individual subunits and function independently.[105,106,107] The physiological relevance of the uncoupled anion conductance is not clear, but an attractive proposal is that the chloride conductance may serve to clamp the membrane potential at a level enabling efficient glutamate accumulation.[36,37] The two positive charges that move into the cell with each transport cycle will depolarize the cell membrane and reduce the driving force for glutamate transport. Entry of chloride ions via the uncoupled chloride conductance is expected to offset the depolarization, enabling the maintance of low extracellular glutamate concentrations. In EAAC1 (also termed EAAT-3[122]), lithium can replace sodium in coupled glutamate uptake but not in its capacity to gate the glutamate-dependent uncoupled anion conductance,[111] and additional studies have reinforced the idea that the conformation gating the anion conductance is different from that during substrate translocation.[123–125] Recent evidence indicates multiple transitions between the coupled transport cycle and anion conductance states.[126] In addition, the uncoupled anion flux can be altered by substituting some of the amino acid residues of transmembrane (TM) domain 2, without significantly affecting the properties of coupled glutamate translocation.[127] Despite these insights, little is known about the mechanism of glutamate-induced anion permeation, but it has been suggested that glutamate itself may gate the anion permeation.[34] Interestingly, it has recently been demonstrated that Glt_{Ph} also exhibits the substrate-modulated anion conductance.[38] Thus, it may be possible to use the Glt_{Ph} structure to aid in probing the mechanism of chloride permeation in the eukaryotic glutamate transporters and to understand the structural basis of a transporter that can act as both a transporter and a channel.

4. Future Directions

The field of neurotransmitter transporters has been transformed by the recently published structures of archeal/bacterial homologues belonging to the SLC1 and SLC6 families. Available functional data on the neurotransmitter transporters indicate that the structures of the homologues are relevant for the study of their eukaryotic counterparts. These structures capture one conformation, and therefore, functional studies of the neurotransmitter transporters, which focus on the conformational changes occurring during transport, are required to close in on the mechanism of neurotransmitter transport. In parallel, high-resolution structures capturing additional conformations will also be of paramount importance toward this long-term goal.

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6. References

(1) Kanner, B. I. *Biochim. Biophys. Acta* **1983**, *726*, 293–316.
(2) Kanner, B. I.; Schuldiner, S. *CRC Crit. Rev. Biochem.* **1987**, *22*, 1–38.
(3) Kanner, B. I. *Curr. Opin. Cell. Biol.* **1989**, *1*, 735–738.
(4) Nelson, N. *J. Neurochem.* **1998**, *71*, 1785–1803.
(5) Giros, B.; Jaber, M.; Jones, S. R.; Wightman, R. M.; Caron, M. G. *Nature* **1996**, *379*, 606–612.
(6) Pines, G.; Danbolt, N. C.; Bjoras, M.; Zhang, Y.; Bendahan, A.; Eide, L.; Koepsell, H.; Storm-Mathisen, J.; Seeberg, E.; Kanner, B. I. *Nature* **1992**, *360*, 464–467.
(7) Tanaka, K.; Watase, K.; Manabe, T.; Yamada, K.; Watanabe, M.; Takahashi, K.; Iwama, H.; Nishikawa, T.; Ichihara, N.; Kikuchi, T.; Okuyama, S.; Kawashima, N.; Hori, S.; Takimoto, M.; Wada, K. *Science* **1997**, *276*, 1699–1702.
(8) Mager, S.; Naeve, J.; Quick, M.; Labarca, C.; Davidson, N.; Lester, H. A. *Neuron* **1993**, *10*, 177–188.

(9) Mager, S.; Kleinberger-Doron, N.; Keshet, G. I.; Davidson, N.; Kanner, B. I.; Lester, H. A. *J. Neurosci.* **1996**, *16*, 5405–5414.
(10) Lester, H. A.; Cao, Y.; Mager, S. *Neuron* **1996**, *17*, 807–810.
(11) Lehre, K. P.; Danbolt, N. C. *J. Neurosci.* **1998**, *18*, 8751–8757.
(12) Chiu, C. S.; Jensen, K.; Sokolova, I.; Wang, D.; Li, M.; Deshpande, P.; Davidson, N.; Mody, I.; Quick, M. W.; Quake, S. R.; Lester, H. A. *J. Neurosci.* **2002**, *22*, 10251–10266.
(13) Yernool, D.; Boudker, O.; Jin, Y.; Gouaux, E. *Nature* **2004**, *431*, 811–818.
(14) Yamashita, A.; Singh, S. K.; Kawate, T.; Jin, Y.; Gouaux, E. *Nature* **2005**, *437*, 215–223.
(15) Boudker, O.; Ryan, R. M.; Yernool, D.; Shimamoto, K.; Gouaux, E. *Nature* **2007**, *445*, 387–393.
(16) Radian, R.; Kanner, B. I. *J. Biol. Chem.* **1985**, *260*, 11859–11865.
(17) Radian, R.; Bendahan, A.; Kanner, B. I. *J. Biol. Chem.* **1986**, *261*, 15437–15441.
(18) Guastella, J.; Nelson, N.; Nelson, H.; Czyzyk, L.; Keynan, S.; Miedel, M. C.; Davidson, N.; Lester, H. A.; Kanner, B. I. *Science* **1990**, *249*, 1303–1306.
(19) Pacholczyk, T.; Blakely, R. D.; Amara, S. G. *Nature* **1991**, *350*, 350–354.
(20) Amara, S. G.; Kuhar, M. J. *Annu. Rev. Neurosci.* **1993**, *16*, 73–93.
(21) Uhl, G. R. *Trends Neurosci.* **1992**, *15*, 265–268.
(22) Storck, T.; Schulte, S.; Hofmann, K.; Stoffel, W. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10955–10959.
(23) Kanai, Y.; Hediger, M. A. *Nature* **1992**, *360*, 467–471.
(24) Jardetzky, O. *Nature* **1966**, *211*, 969–970.
(25) Olesen, C.; Picard, M.; Winther, A. M.; Gyrup, C.; Morth, J. P.; Oxvig, C.; Moller, J. V.; Nissen, P. *Nature* **2007**, *450*, 1036–1042.
(26) Kavanaugh, M. P.; Bendahan, A.; Zerangue, N.; Zhang, Y.; Kanner, B. I. *J. Biol. Chem.* **1997**, *272*, 1703–1708.
(27) Zhang, Y.; Bendahan, A.; Zarbiv, R.; Kavanaugh, M. P.; Kanner, B. I. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 751–755.
(28) Keynan, S.; Suh, Y. J.; Kanner, B. I.; Rudnick, G. *Biochemistry* **1992**, *31*, 1974–1979.
(29) Roux, M. J.; Supplisson, S. *Neuron* **2000**, *25*, 373–383.
(30) Loo, D. D.; Hazama, A.; Supplisson, S.; Turk, E.; Wright, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5767–5771.
(31) Wadiche, J. I.; Arriza, J. L.; Amara, S. G.; Kavanaugh, M. P. *Neuron* **1995**, *14*, 1019–1027.
(32) Mager, S.; Min, C.; Henry, D. J.; Chavkin, C.; Hoffman, B. J.; Davidson, N.; Lester, H. A. *Neuron* **1994**, *12*, 845–859.
(33) Sonders, M. S.; Zhu, S. J.; Zahniser, N. R.; Kavanaugh, M. P.; Amara, S. G. *J. Neurosci.* **1997**, *17*, 960–974.
(34) Wadiche, J. I.; Amara, S. G.; Kavanaugh, M. P. *Neuron* **1995**, *15*, 721–728.
(35) Fairman, W. A.; Vandenberg, R. J.; Arriza, J. L.; Kavanaugh, M. P.; Amara, S. G. *Nature* **1995**, *375*, 599–603.
(36) Billups, B.; Rossi, D.; Attwell, D. *J. Neurosci.* **1996**, *16*, 6722–6731.
(37) Eliasof, S.; Jahr, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4153–4158.
(38) Ryan, R. M.; Mindell, J. A. *Nat. Struct. Mol. Biol.* **2007**, *14*, 365–371.
(39) Chen, J. G.; Liu-Chen, S.; Rudnick, G. *J. Biol. Chem.* **1998**, *273*, 12675–12681.
(40) Toyoshima, C.; Nakasako, M.; Nomura, H.; Ogawa, H. *Nature* **2000**, *405*, 647–655.
(41) Hama, H.; Wilson, T. H. *J. Biol. Chem.* **1993**, *268*, 10060–10065.
(42) Zhang, Y.; Kanner, B. I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1710–1715.
(43) Androutsellis-Theotokis, A.; Goldberg, N. R.; Ueda, K.; Beppu, T.; Beckman, M. L.; Das, S.; Javitch, J. A.; Rudnick, G. *J. Biol. Chem.* **2003**, *278*, 12703–12709.
(44) Quick, M.; Yano, H.; Goldberg, N. R.; Duan, L.; Beuming, T.; Shi, L.; Weinstein, H.; Javitch, J. A. *J. Biol. Chem.* **2006**, *281*, 26444–26454.
(45) Keynan, S.; Kanner, B. I. *Biochemistry* **1988**, *27*, 12–17.
(46) Forrest, L. R.; Tavoulari, S.; Zhang, Y. W.; Rudnick, G.; Honig, B. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12761–12766.
(47) Zomot, E.; Bendahan, A.; Quick, M.; Zhao, Y.; Javitch, J. A.; Kanner, B. I. *Nature* **2007**, *449*, 726–730.
(48) Kilic, F.; Rudnick, G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3106–3111.
(49) Scholze, P.; Freissmuth, M.; Sitte, H. H. *J. Biol. Chem.* **2002**, *277*, 43682–43690.
(50) Hastrup, H.; Karlin, A.; Javitch, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10055–10060.
(51) Hastrup, H.; Sen, N.; Javitch, J. A. *J. Biol. Chem.* **2003**, *278*, 45045–45048.
(52) Bismuth, Y.; Kavanaugh, M. P.; Kanner, B. I. *J. Biol. Chem.* **1997**, *272*, 16096–16102.
(53) Chen, J. G.; Sachpatzidis, A.; Rudnick, G. *J. Biol. Chem.* **1997**, *272*, 28321–28327.
(54) Ponce, J.; Biton, B.; Benavides, J.; Avenet, P.; Aragon, C. *J. Biol. Chem.* **2000**, *275*, 13856–13862.
(55) Kanner, B. I. *J. Biol. Chem.* **2003**, *278*, 3705–3712.
(56) Beuming, T.; Shi, L.; Javitch, J. A.; Weinstein, H. *Mol. Pharmacol.* **2006**, *70*, 1630–1642.
(57) Barker, E. L.; Moore, K. R.; Rakhshan, F.; Blakely, R. D. *J. Neurosci.* **1999**, *19*, 4705–4717.
(58) Rees, M. I.; Harvey, K.; Pearce, B. R.; Chung, S. K.; Duguid, I. C.; Thomas, P.; Beatty, S.; Graham, G. E.; Armstrong, L.; Shiang, R.; Abbott, K. J.; Zuberi, S. M.; Stephenson, J. B.; Owen, M. J.; Tijssen, M. A.; van den Maagdenberg, A. M.; Smart, T. G.; Supplisson, S.; Harvey, R. J. *Nat. Genet.* **2006**, *38*, 801–806.
(59) Vandenberg, R. J.; Shaddick, K.; Ju, P. *J. Biol. Chem.* **2007**, *282*, 14447–14453.
(60) Dodd, J. R.; Christie, D. L. *J. Biol. Chem.* **2007**, *282*, 15528–15533.
(61) Zhou, Y.; Zomot, E.; Kanner, B. I. *J. Biol. Chem.* **2006**, *281*, 22092–22099.
(62) Kavanaugh, M. P.; Arriza, J. L.; North, R. A.; Amara, S. G. *J. Biol. Chem.* **1992**, *267*, 22007–22009.
(63) Lu, C. C.; Hilgemann, D. W. *J. Gen. Physiol.* **1999**, *114*, 429–444.
(64) Bennett, E. R.; Su, H.; Kanner, B. I. *J. Biol. Chem.* **2000**, *275*, 34106–34113.
(65) MacAulay, N.; Zeuthen, T.; Gether, U. *J. Physiol.* **2002**, *544*, 447–458.
(66) Talvenheimo, J.; Fishkes, H.; Nelson, P. J.; Rudnick, G. *J. Biol. Chem.* **1983**, *258*, 6115–6119.
(67) Quick, M. W. *Neuron* **2003**, *40*, 537–549.
(68) Hilgemann, D. W.; Lu, C. C. *J. Gen. Physiol.* **1999**, *114*, 459–475.
(69) Krause, S.; Schwarz, W. *Mol. Pharmacol.* **2005**, *68*, 1728–1735.
(70) Accardi, A.; Miller, C. *Nature* **2004**, *427*, 803–807.
(71) Dutzler, R.; Campbell, E. B.; Cadene, M.; Chait, B. T.; MacKinnon, R. *Nature* **2002**, *415*, 287–294.
(72) Dutzler, R.; Campbell, E. B.; MacKinnon, R. *Science* **2003**, *300*, 108–112.
(73) Pantanowitz, S.; Bendahan, A.; Kanner, B. I. *J. Biol. Chem.* **1993**, *268*, 3222–3225.
(74) Mari, S. A.; Soragna, A.; Castagna, M.; Santacroce, M.; Perego, C.; Bossi, E.; Peres, A.; Sacchi, V. F. *Cell. Mol. Life Sci.* **2006**, *63*, 100–111.
(75) Loland, C. J.; Granas, C.; Javitch, J. A.; Gether, U. *J. Biol. Chem.* **2004**, *279*, 3228–3238.
(76) Singh, S. K.; Yamashita, A.; Gouaux, E. *Nature* **2007**, *448*, 952–956.
(77) Zhou, Z.; Zhen, J.; Karpowich, N. K.; Goetz, R. M.; Law, C. J.; Reith, M. E.; Wang, D. N. *Science* **2007**, *317*, 1390–1393.
(78) Rudnick, G. *ACS Chem. Biol.* **2007**, *2*, 606–609.
(79) Zomot, E.; Kanner, B. I. *J. Biol. Chem.* **2003**, *278*, 42950–42958.
(80) Mitchell, S. M.; Lee, E.; Garcia, M. L.; Stephan, M. M. *J. Biol. Chem.* **2004**, *279*, 24089–24099.
(81) MacAulay, N.; Bendahan, A.; Loland, C. J.; Zeuthen, T.; Kanner, B. I.; Gether, U. *J. Biol. Chem.* **2001**, *276*, 40476–40485.
(82) Mabjeesh, N. J.; Kanner, B. I. *Biochemistry* **1993**, *32*, 8540–8546.
(83) Henry, L. K.; Adkins, E. M.; Han, Q.; Blakely, R. D. *J. Biol. Chem.* **2003**, *278*, 37052–37063.
(84) Zhou, Y.; Bennett, E. R.; Kanner, B. I. *J. Biol. Chem.* **2004**, *279*, 13800–13808.
(85) Chen, J. G.; Rudnick, G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1044–1049.
(86) Golovanevsky, V.; Kanner, B. I. *J. Biol. Chem.* **1999**, *274*, 23020–23026.
(87) Zhang, Y. W.; Rudnick, G. *J. Biol. Chem.* **2006**, *281*, 36213–36220.
(88) Jiang, Y.; Lee, A.; Chen, J.; Cadene, M.; Chait, B. T.; MacKinnon, R. *Nature* **2002**, *417*, 523–526.
(89) Weinglass, A. B.; Smirnova, I. N.; Kaback, H. R. *Biochemistry* **2001**, *40*, 769–776.
(90) Zhou, Y.; Kanner, B. I. *J. Biol. Chem.* **2005**, *280*, 20316–20324.
(91) Kanner, B. I.; Sharon, I. *Biochemistry* **1978**, *17*, 3949–3953.
(92) Brew, H.; Attwell, D. *Nature* **1987**, *327*, 707–709.
(93) Zerangue, N.; Kavanaugh, M. P. *Nature* **1996**, *383*, 634–637.
(94) Levy, L. M.; Warr, O.; Attwell, D. *J. Neurosci.* **1998**, *18*, 9620–9628.
(95) Kanner, B. I.; Bendahan, A. *Biochemistry* **1982**, *21*, 6327–6330.
(96) Pines, G.; Kanner, B. I. *Biochemistry* **1990**, *29*, 11209–11214.
(97) Watzke, N.; Bamberg, E.; Grewer, C. *J. Gen. Physiol.* **2001**, *117*, 547–562.
(98) Bergles, D. E.; Tzingounis, A. V.; Jahr, C. E. *J. Neurosci.* **2002**, *22*, 10153–10162.
(99) Arriza, J. L.; Eliasof, S.; Kavanaugh, M. P.; Amara, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4155–4160.
(100) Grunewald, M.; Bendahan, A.; Kanner, B. I. *Neuron* **1998**, *21*, 623–632.
(101) Slotboom, D. J.; Sobczak, I.; Konings, W. N.; Lolkema, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14282–14287.

(102) Grunewald, M.; Kanner, B. I. *J. Biol. Chem.* **2000**, *275*, 9684–9689.
(103) Groeneveld, M.; Slotboom, D. J. *J. Mol. Biol.* **2007**, *372*, 565–570.
(104) Koch, H. P.; Larsson, H. P. *J. Neurosci.* **2005**, *25*, 1730–1736.
(105) Grewer, C.; Balani, P.; Weidenfeller, C.; Bartusel, T.; Tao, Z.; Rauen, T. *Biochemistry* **2005**, *44*, 11913–11923.
(106) Leary, G. P.; Stone, E. F.; Holley, D. C.; Kavanaugh, M. P. *J. Neurosci.* **2007**, *27*, 2938–2942.
(107) Koch, H. P.; Brown, R. L.; Larsson, H. P. *J. Neurosci.* **2007**, *27*, 2943–2947.
(108) Brocke, L.; Bendahan, A.; Grunewald, M.; Kanner, B. I. *J. Biol. Chem.* **2002**, *277*, 3985–3992.
(109) Bendahan, A.; Armon, A.; Madani, N.; Kavanaugh, M. P.; Kanner, B. I. *J. Biol. Chem.* **2000**, *275*, 37436–37442.
(110) Shimamoto, K.; Lebrun, B.; Yasuda-Kamatani, Y.; Sakaitani, M.; Shigeri, Y.; Yumoto, N.; Nakajima, T. *Mol. Pharmacol.* **1998**, *53*, 195–201.
(111) Borre, L.; Kanner, B. I. *J. Biol. Chem.* **2001**, *276*, 40396–40401.
(112) Teichman, S.; Kanner, B. I. *J. Gen. Physiol.* **2007**, *129*, 527–539.
(113) Rosental, N.; Bendahan, A.; Kanner, B. I. *J. Biol. Chem.* **2006**, *281*, 27905–27915.
(114) Ogawa, H.; Toyoshima, C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15977–15982.
(115) Tao, Z.; Zhang, Z.; Grewer, C. *J. Biol. Chem.* **2006**, *281*, 10263–10272.
(116) Tao, Z.; Grewer, C. *J. Gen. Physiol.* **2007**, *129*, 331–344.
(117) Menaker, D.; Bendahan, A.; Kanner, B. I. *J. Neurochem.* **2006**, *99*, 20–28.
(118) Grunewald, M.; Menaker, D.; Kanner, B. I. *J. Biol. Chem.* **2002**, *277*, 26074–26080.
(119) Shlaifer, I.; Kanner, B. I. *Mol. Pharmacol.* **2007**, *71*, 1341–1348.
(120) Zarbiv, R.; Grunewald, M.; Kavanaugh, M. P.; Kanner, B. I. *J. Biol. Chem.* **1998**, *273*, 14231–14237.
(121) Wadiche, J. I.; Kavanaugh, M. P. *J. Neurosci.* **1998**, *18*, 7650–7661.
(122) Arriza, J. L.; Fairman, W. A.; Wadiche, J. I.; Murdoch, G. H.; Kavanaugh, M. P.; Amara, S. G. *J. Neurosci.* **1994**, *14*, 5559–5569.
(123) Borre, L.; Kavanaugh, M. P.; Kanner, B. I. *J. Biol. Chem.* **2002**, *277*, 13501–13507.
(124) Ryan, R. M.; Vandenberg, R. J. *J. Biol. Chem.* **2002**, *277*, 13494–13500.
(125) Seal, R. P.; Shigeri, Y.; Eliasof, S.; Leighton, B. H.; Amara, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 15324–15329.
(126) Shachnai, L.; Shimamoto, K.; Kanner, B. I. *Neuropharmacology* **2005**, *49*, 862–871.
(127) Ryan, R. M.; Mitrovic, A. D.; Vandenberg, R. J. *J. Biol. Chem.* **2004**, *279*, 20742–20751.

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