Slips, leaks and channels in glutamate transporters

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Abbreviations: EAAT, human excitatory amino acid transporter; GLT-1, rat glutamate transporter 1 (homolog of human EAAT2); TM, transmembrane domain; HP, hairpin loop; TBOA, DL-threo-benzoyloxyaspartate

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Glutamate transporters are unusual proteins in that they can function as both a transporter and a chloride channel. With the recent determination of the crystal structure of an archaeal aspartate transporter it is now possible to begin to put together a physical picture of how these proteins are able to carry out their dual functions. In this review we shall discuss our current understanding of the functional states of glutamate transporters and how they may arise. We will also discuss some of the alternate conducting states of glutamate transporters and provide definitions of the various states.

Introduction

Ion channels and secondary active transporters have traditionally been classified as two distinct types of membrane proteins. Channels can be thought of as proteins that consist of binding sites located along a transmembrane pathway that may be accessible from both sides of the membrane at the same time, whereas transporters allow substrate and ion binding sites to be exposed alternately to both sides of the membrane.1 Opening of an ion channel will allow ions to flow through an aqueous pore in the membrane and dissipate the electrochemical gradient of the permeant ion where as transporters utilize the gradient of one or more ionic species to drive the movement of another solute against its concentration gradient. This has been an important distinction because for secondary active transporters the number of co- or counter-transported ions coupled to the transport process determines the concentrating capacity of the transporter2 and any changes to the coupling process will impact on the concentrating capacity. However, over the last 20–30 years, the distinctions between channels and transporters have become somewhat blurred. There are examples of: transporters that have ion channel functions;3-5 transporters that allow ions to leak through the membrane;6 channels that have ion flux coupling7 and membrane proteins that were initially thought of as ion channels that behave as transporters.8 Indeed, it was a prophetic statement by one of the pioneers of the transporter field, Peter Lauger that “channel and transporter models should not be viewed as mutually exclusive possibilities, but rather as limiting cases of a more general mechanism”.1 The study of channels and transporters has received enormous impetus in the last 5-10 years with the determination of the crystal structures of a number of transporters and ion channels and in this review we will discuss some of the emerging concepts for the structural basis for transporter functions and how transporters can function as ion channels. Glutamate transporters are a particularly interesting class of transporter/ ion channels and will be the focus of this review. Glutamate transporters are very powerful transporters, but in addition to their transport function they can act as ligand-gated Cl- channels.3 We will discuss some of the recent efforts to begin to understand the structural basis for the different conducting states of glutamate transporters and in so doing begin to explore the similarities and differences between ion channels and transporters and how these processes can influence cellular biology.

Ion-Flux Coupling in Glutamate Transporters

The stoichiometry of ion-flux coupling for the mammalian glutamate transporter EAAT3 has been very elegantly studied by Zerangue and Kavanaugh2 by measuring the ratio of reversal potential changes whilst systematically altering the ion gradients of the coupled ions Na+, K+ and H+. Using this procedure, the ion-flux coupling ratios were estimated to be 3 Na+ and 1H+ co-transported with each glutamate, followed by the counter-transport of 1 K+ (Fig. 1). After taking into account the net -1 charge on the glutamate molecule, the total charge transfer across the membrane is two positive charges per transport cycle. From these ratios, the concentrating capacity of the transporter can be estimated using a modified version of the Goldman Hodgkin Katz equation:

$$\frac{[\text{Glu}]}{[\text{Glu}]} = \frac{RT}{ZF} \ln \left( \frac{[\text{Na}^+]}{[\text{Na}^+]} \cdot \frac{[\text{K}^+]}{[\text{K}^+]} \right)$$

where, [X]o and [X]i refer to the outside and inside concentrations of the various ionic species, R is the gas constant, T is temperature in °K, F is Faraday’s constant. Z = 2 because there is a net transfer of two positive charges. Note that the term referring to the Na+ gradient is to the power of three because 3 Na+ ions are coupled to the transport...
process. A similar study has been carried out with the rat equivalent of EAAT3 concludes that this residue is not involved in Na+ binding. 22,23 Clearly further experiments are required to clarify the location of the Na+ binding sites in GltPh and the EAATs. DL-threo-β-benzyloxyaspartate (TBOA) is a non-transportable blocker of the EAATs and also inhibits aspartate transport by GltPh (Ryan and Mindell, unpublished results).

Figure 1. Stoichiometry of Ion-Flux coupling. Glutamate transport by the EAATs is coupled to the co-transport of 3 Na+ and 1 H+, following by the counter-transport of 1 K+. In addition, glutamate and Na+ binding to the transporter activates an uncoupled Cl- conductance. Aspartate transport by GltPh is coupled to the co-transport of at least 2 Na+. The transport process also activates an uncoupled Cl- conductance in GltPh.

Structure of an Archaeal Glutamate Transporter Homolog

The crystal structure of an archaeal aspartate transporter, GltPh, has recently been determined11 and its amino acid sequence shows considerable homology with the mammalian glutamate transporters. Approximately 36% of amino acid residues are conserved between the archaeal and mammalian transporters and the degree of identity is considerably higher in regions that have been implicated in playing important functional roles in the transport process. Thus, the GltPh structure is considered a very good model for the structures of the mammalian glutamate transporters. In agreement with biochemical studies on the mammalian glutamate transporters, the crystal structure of GltPh is a trimer.11-13 Three protomers come together to form an extracellular facing bowl shaped complex. The basin of the bowl reaches almost halfway across the membrane while the intracellular portions of each protomer come together to form a pointed wedge structure (Fig. 2A). The individual protomers contain a number of unusual secondary structure features; there are eight transmembrane (TM) domains that vary in length from 19 to 49 residues and two helix-turn-helix motifs or hairpin loops termed HP1 and HP2 (Fig. 2B). The carboxy-terminal half of each protomer, composed of HP1, HP2, TM7 and TM8 is the most highly conserved region between the archaeal and mammalian homologues and is encased in a shell consisting of the amino-terminal half of each protomer.

GltPh is a Na+-dependent transporter that exhibits selectivity for aspartate over glutamate.14 This is in contrast to the EAATs, which transport L-glutamate and L-,D-aspartate with similar affinities. In the original structure of GltPh, a non-protein electron density was observed in close proximity to the tips of HP1 and HP2 and to portions of TM7 and TM8 and it was proposed that this region was the substrate binding domain.11 As the current resolution of the GltPh structure (~3 Å) does not permit the direct visualization of bound ions and substrate, an analogue of aspartate which binds with similar affinity was used to probe the substrate binding site. L-cysteine sulfinic acid contains a sulfur atom, which has anomalous scattering properties that allows us to see this compound in the GltPh structure. This technique confirmed that aspartate binds in the predicted substrate binding domain which is a highly polar chamber located almost halfway across the membrane14 (Fig. 2C). Many of the residues that have been implicated in substrate and coupled ion binding/transport in the EAATs, based on mutagenesis studies,15-18 are found in this substrate binding domain and interact with bound aspartate. Thus, both X-ray crystallography and mutagenesis of the EAATs yielded similar results in terms of the residues required for substrate interactions. Each protomer contains its own substrate recognition site, which implies that each protomer is capable of substrate transport.11 This structural data agrees well with many functional studies on the EAATs that demonstrate that each individual protomer is capable of substrate transport and Cl- flux19-21 (see below).

GltPh has an absolute requirement for Na+; at least two Na+ ions are coupled to the binding14 and transport (Ryan and Mindell, unpublished results) of each aspartate molecule (Fig. 1). Crystallography studies employing the heavy atom thallium (Tl+) identified 2 Na+ selective ion binding sites.14 At this stage it is not clear if there is a third Na+ ion coupled to GltPh that is not replaced by Tl+ and thus not identified in the crystal structure or if in fact GltPh is only coupled to two Na+ ions. Na+ site #1 is formed by elements of TM7 and TM8 and sits below bound substrate while Na+ site #2 is coordinated by HP2a and TM7a and appears to lock HP2 over the substrate binding domain (Fig. 2C). All of the contacts for site #2 are formed by main chain interactions and only one side chain carboxylate of residue D405 appears to coordinate the binding of the Na+ ion at site #1. The mutation of D405 to an asparagine residue in GltPh reduces the coupling of Na+ to aspartate binding and also results in the loss of the ion binding site #1 in the crystal structure.14 D405 is highly conserved in all the known Na+-dependent transporters of this family, but is replaced by an asparagine residue in the known H+-dependent transporters. In contrast to these results, a recent study investigating the role of the equivalent aspartate residue in EAAT3 concludes that this residue is not involved in Na+ binding to EAAT3 and other residues in TM7 and TM8 were implicated in Na+ binding.22,23 Clearly further experiments are required to clarify the location of the Na+ binding sites in GltPh and the EAATs. It is also interesting to note that K+ and H+ do not appear to be coupled to the transport of aspartate by GltPh (Ryan and Mindell, unpublished results).

DL-threo-β-benzyloxyaspartate (TBOA) is a non-transportable blocker of the EAATs24 and also inhibits aspartate transport by GltPh. The structure of GltPh in complex with TBOA has been solved and compared to the aspartate bound structure.14 Overall these structures are very similar, but there are two important differences; HP2 moves away from the substrate binding site and makes direct contacts with the extracellular loop between TM3 and TM4. The aspartate moiety of TBOA binds in the aspartate binding site, but the benzyl ring of
takes place. One aspect of the structure that is envisaged as playing an important role is HP1. For example, it will be of interest to understand how the movements of HP2 and HP1 are coordinated during the transport process, and whether this plays a role in the coupling process. Furthermore, it will be of interest to understand how conformational changes in the key regions are required for K\(^+\) translocation in the EAATs.

**Uncoupled Ion Movements in Glutamate Transporters**

The previous sections have discussed the structural basis for the defining feature of glutamate transporters, namely the coupling of downhill movement of Na\(^+\) to the uphill movement of glutamate. In the following sections we will discuss a variety of alternate conducting states of glutamate transporters, which have begun to blur the distinction between transporters and ion channels. These alternate conducting states may have: a direct bearing on the functional capacity of the transporter; or impact on the cellular biology of the cells in which they are expressed; or may simply be a biochemical consequence of a powerful and efficient transport system. Irrespective of the biological outcomes of these alternate conducting states, their existence has implications for how these proteins work and how we define a transporter.

A number of terms have been used to describe the non-classical conducting states of transporters and there is some overlap in the definitions used for different transporters. As glutamate transporters have a number of different conducting states, it is important to define what each state represents. The rationale that we have taken is to use terms that provide a mechanistic insight. The first term, which is probably the most interesting and to some extent the most controversial, is the “substrate-activated chloride conductance”.

In the case of glutamate transporters, glutamate and Na\(^+\) binding to the transporter activates an intrinsic Cl\(^-\) conductance, which for some transporter subtypes can allow a significant Cl\(^-\) flux (Fig. 3). Some of the reasons why this current has generated such interest and controversy are: Can it influence the biology of the cell? Is it a term that has been used to describe the situation where additional ions pass through the transporter that are not coupled to the transport process. Although there is no single definition of slippage that covers all cases of this phenomenon, the one that we find most useful is where the additional ions that pass through the transporter are the
The rate of glutamate transport is independent of Cl⁻ ion concentration. These observations demonstrate that Cl⁻ ion movement through the transporter itself through the transporter (Fig. 3).

**3.2 Cl⁻ Channel Activity in Glutamate Transporters**

In 1988, Attwell’s group described a Cl⁻ conductance in bipolar cells of the retina that required both glutamate and Na⁺ for activation, but was distinct from other ionotropic glutamate receptors. The requirement for both glutamate and Na⁺ suggested that the Cl⁻ conductance was in some way associated with glutamate transporters. A number of other studies made similar observations, but it was not clear at this stage whether the Cl⁻ conductance was mediated by a separate Cl⁻ channel that closely associates with glutamate transporters or if the glutamate transporter itself mediated the Cl⁻ conductance. This issue was resolved in a study by Fairman et al. with the characterization of the EAAT4 glutamate transporter. The characteristics of this transporter coincided closely with the properties observed by Sarantis et al. The cloning and expression of glutamate transporters in cells has greatly facilitated the study of these additional conductance states and also the structural basis for these functions. Application of glutamate to cells expressing the recombinant EAAT4 generates a current that is predominantly due to Cl⁻ ions, which reverses direction at the Cl⁻ reversal potential. At membrane potentials both above and below the Cl⁻ reversal potential there is a Na⁺-dependent uptake of 3H-glutamate. Furthermore, the rate of glutamate transport is independent of Cl⁻ ion concentration. These observations demonstrate that Cl⁻ ion movement through the transporter is thermodynamically uncoupled from the transport process. Subsequent studies of EAAT1, 2, 3 (ref. 4) and 5 (ref. 28) confirmed that all of the mammalian glutamate transporters have an intrinsic Cl⁻ conductance. The magnitude of the Cl⁻ conductance relative to the coupled transport process varies between the transporter subtypes, being greatest for EAAT4 and EAAT5 followed by EAAT1, EAAT3 and EAAT2. Wadiche and Kavanagh have gone on further to characterize the properties of the glutamate-activated Cl⁻ conductance and demonstrated that the Cl⁻ conductance has a Q₁₀ of 1.0 ± 0.1 whereas the Q₁₀ for the transport process is 3.2 ± 0.2, which suggests that although the processes are linked the energy required for activation or movement of Cl⁻ ions through the transporter is less than that for the coupled transport process. The Q₁₀ for activation of the Cl⁻ conductance is what is expected of ionic diffusion through an aqueous solution and as such is more akin to a channel mechanism than a transport process. It is also interesting to note that glutamate transporters also allow water molecules to pass through at a rate that is comparable to aquaporins. At this stage it has not been determined whether the water molecules pass through the transporter associated with the transport process or associated with the uncoupled Cl⁻ movement. The amino acid residues that influence Cl⁻ selectivity are aqueous accessible (see below), and so it is likely that water molecules will pass through the Cl⁻ permeation pathway. So, on these grounds the Cl⁻ conductance of glutamate transporters is likely to be mediated by a water filled pore, which is in line with the definition of a channel.

Although Cl⁻ is the predominant physiological anion, other anions can also permeate the EAATs in an uncoupled manner. The relative anion permeabilities compared to Cl⁻ are: SCN⁻ (67-fold more permeant than Cl⁻) > NO₃⁻ (17) > I⁻ (12) > Br⁻ (3) > Cl⁻ (1) >> F⁻, gluconate, methanesulfonate. From these measurements the minimum pore diameter is estimated to be ~5 Å. Wadiche and Kavanagh also attempted to measure single channel currents from patches pulled from Xenopus laevis oocytes expressing EAAT1, but were unable to detect single channel events. However, from noise analysis, they were able to estimate the single channel conductance in Cl⁻ to be ~1fS, with an open probability of <1%. It has recently been demonstrated that the arachidial aspartate transporter, Glt1p, also has an intrinsic substrate-activated Cl⁻ conductance with similar properties to the uncoupled Cl⁻ conductance of the EAATs. Thus, it appears that the Cl⁻ conductance of these transporters is a function that has been retained through evolution, and implies that, at least under some circumstances, it plays an important biological/mechanistic role.

**Leaks and Slips in Glutamate Transporters**

In the absence of glutamate, cells expressing glutamate transporters have a small Na⁺ dependent Cl⁻ leak conductance that can be detected with the use of glutamate transport blockers, such as TBOA (Fig. 3). Under standard physiological conditions the amplitude of the leak conductance is ~1-5% of the substrate-activated Cl⁻ conductance, with its magnitude varying with the different glutamate transporter subtypes. It is possible to enhance the magnitude of the conductance by using NO₃⁻ or other more permeant anions, but it is interesting to note that the relative anion permeability characteristics of the leak currents differ from the substrate activated anion currents. The relative anion permeability sequence for the leak in EAAT1 is I⁻ (2.3) > NO₃⁻ (2.0) > Br⁻ (1.2) > Cl⁻ (1.0). Although both the substrate-activated anion conductance and the leak anion conductance are mediated by the same protein, the selectivity filters for the two conductance states must be different. At this stage it is not clear whether these filters are physically distinct or different conformational states of a common structural motif.

Co-application of arachidonic acid with glutamate to oocytes expressing the EAAT4 transporter generates an uncoupled H⁺ conductance. Furthermore, co-application of the cyclooxygenase inhibitor, niflumic acid, with either L-aspartate or glutamate also generates an additional H⁺ flux through the transporter.
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A number of physiological functions have been proposed for the Cl\(^-\) channel of glutamate transporters. As the magnitude of the Cl\(^-\) conductance varies between transporter subtypes, it is likely that the Cl\(^-\) conductance will serve different functions in the different cells in which they are expressed. The first and simplest function that has been proposed for these channels is as a charge neutralization mechanism or maintaining ionic homeostasis. Coupled glutamate transport generates a net transfer of two positive charges per transport cycle; an additional Cl\(^-\) flux via the transporter may alleviate this build up of positive charge. Whilst the amount of charge transfer that will occur for a single transporter is negligible, in glial cells surrounding glutamatergic synapses, glutamate transporter proteins are highly abundant. It has been estimated that the number of transporters is comparable to the number of glutamate molecules released from a synaptic vesicle. Therefore, after a couple of cycles of the large number of the transporters, there is potential for depolarization of these cells, which would feedback to inhibit further transport. This process is dependent on the expression levels of the transporters and also the intracellular volume. In the case of the bacterial aspartate transporter, GltPh, expressed in liposomes, if permeant anions are not present then the rate of transport slows very rapidly and effectively prevents sustained aspartate transport. If Cl\(^-\) or other permeant anions are present in the media, the transport is maintained over a much longer period. This phenomenon has not been directly demonstrated for the mammalian glutamate transporters, but it is anticipated that such a process would occur where there is abundant glutamate transporter expression, especially in small glial processes that come in close contact with the synapse.

The human glutamate transporters EAAT4 and EAAT5 are expressed in neurons and have relatively large Cl\(^-\) conductances compared to the transport conductance,\(^5,28\) which provides the intriguing possibility that they may play a signaling role. In a very elegant report, Veruki et al.\(^{41}\) studied the role of the Cl\(^-\) conductance of glutamate transporters in regulation of synaptic transmission between rod bipolar cells and amacrine cells. Activation of the Cl\(^-\) conductance associated with glutamate transporters in the presynaptic rod bipolar cells caused hyperpolarization which then reduced the probability of subsequent glutamate release. Thus, the substrate-activated Cl\(^-\) conductance of the transporter has the capacity to reduce glutamate levels in these synapses, but in a fundamentally different manner to that for standard ion-coupled transport. It is interesting to note that in the sodium and chloride-dependent neurotransmitter transporter family, the dopamine transporter also supports a substrate-activated Cl\(^-\) conductance. In this case, the chloride conductance of the presynaptic dopamine transporter feeds back to influence membrane potential, but in contrast to glutamatergic neurons, the dopaminergic neurons are depolarized leading to enhancement of dopamine release.\(^{42}\)

Another suggestion for the existence of a Cl\(^-\) conductance in glutamate transporters is that it is a by-product of the transport...
mechanism. The structure of glutamate transporters appears to be ideal for allowing fast and efficient transport. Glutamate diffuses half way across the membrane before coming in contact with a substrate binding site which is made up of HP1 and HP2 and also TM7 and TM8 \(^{11,14}\) (see above). HP1 and HP2 are likely to undergo considerable conformational changes during the transport process and this is made possible by encasing this working part of the protein within a pseudo-cylindrical structure that may facilitate these movements. However, a consequence of the conformational changes in HP1 and HP2 and possibly TM7 and TM8 may be that Cl\(^-\) ions may pass between the working inner part of the protein and the outer shell (Fig. 4). Thus, the Cl\(^-\) conductance may be simply a by-product of the transport mechanism.

**The Structural Basis for Cl\(^-\) Channel Function**

In three separate studies, \(^{43-45}\) it was demonstrated that changing residues in HP2 to cysteine and modification with the sulphhydryl reactive reagent methane \([2-(trimethyl-ammonium) ethyl]methyl ethanethiosulfonate\) allowed glutamate binding and activation of the Cl\(^-\) conductance, but prevented glutamate transport. One conclusion that may be drawn from these studies is that there are different conformational states required for transport compared to activation of the Cl\(^-\) conductance and that there are likely to be distinct molecular determinants for the two processes. In a follow up study, Ryan et al., \(^{21}\) identified a series of residues in TM2 that influence Cl\(^-\) permeability and also gating of the Cl\(^-\) conductance, but do not influence glutamate transport. In particular, mutation of S103 to V changed the order of relative anion permeability such that Br\(^-\) is more permeant than NO\(_3\)\(^-\), which is indicative of a change in the interactions between anions and the pore of the channel and implies that this residue influences the selectivity filter. This part of TM2 is accessible to the aqueous solution as judged by reactivity of the S103C mutant to HgCl\(_2\) and as such may be part of a water filled pore. In Gltp\(_b\), the equivalent residue is S65 (Fig. 4) and the S65V mutant shows a comparable disruption to Cl\(^-\) channel function as the S103V mutation in EAAT1. \(^{32}\) Thus, it appears that the molecular determinants for Cl\(^-\) selectivity are conserved between the human EAATs and Gltp\(_b\). At the intracellular edge of TM2 in EAAT1 is D112 and mutation of this residue to A results in a functional glutamate transporter but changes the gating properties of the Cl\(^-\) conductance. Application of the glutamate transport blocker, TBOA, to cells expressing glutamate transporters reveals a leak current. The amplitude of the leak current in the D112A mutant is about 20-fold greater than the leak current present in wild type EAAT1, but the anion permeability characteristics of this leak are similar to the wild type leak. Furthermore, application of glutamate or aspartate to the D112A mutant transporter does not generate an additional uncoupled anion conductance. From these observations it was concluded that the D112A mutation locks the anion channel in an open state and application of substrate does not activate any further anion conductance. One interpretation of these results is that the leak and substrate-gated anion conductance pathways are very closely related and the differences in relative anion permeability between the two conductances arise as a subtle alteration in the conformation of the selectivity filter. However, an equally plausible conclusion is that the D112A mutation facilitates opening of the leak conductance pathway, but prevents opening of a separate substrate-activated anion pathway. This implies that the pathways are separate with different selectivity filters and different gating mechanisms. Our group has attempted to identify further residues that may be involved in gating of the leak and substrate-activated anion channel by using a homology model of EAAT1, based on the structure of Gltp\(_b\). \(^{34}\) We investigated charged residues that were within 5Å of D112 and thus could influence the conformation of D112. Mutations of D272 in TM5 and K384 and R385 in TM7 to neutral or opposite charges also generated changes in both the uncoupled chloride conductance and the leak conductance, but the nature of the changes were different to that of the D112A mutant. In contrast to the D112A transporter, the amplitudes of the substrate-activated uncoupled Cl\(^-\) conductance of the D272A, K384D and R385D mutants were significantly greater than wild type EAAT1 with little, if any, changes in relative anion permeability. The amplitudes of the leak currents were also significantly greater for the D272A, D272K, K384A and R385A mutants, and again with no changes in relative anion permeabilities. Therefore, these residues are unlikely to form part of the selectivity filter for either the uncoupled Cl\(^-\) conductance or the leak conductance, but they do play roles in gating of the two conductances. D272 is located in TM5, and either neutralization or opposite polarity mutations increase the amplitude of both the leak and substrate-activated channel conductance. On the other hand, neutralization of K384 or R385 does not appear to alter gating of the substrate-activated anion conductance, whereas opposite polarity mutations increase the amplitude of both the leak and substrate-activated channel conductances. Thus, both the position and charge of residues in this intracellular region of EAAT1 play key roles in determining the gating properties of the two conductances and it would appear that there are residues that differentially affect gating of one process over the other. Does this mean that there are separate gates for the two conductance states? Furthermore, are the permeation pathways the same? At this stage we cannot answer these questions.

The identification of residues, K384 and R385, at the intracellular edge of TM7 as playing a role in gating of the two conductances does provide a provocative suggestion as to how the substrate and ions may play a role in the gating process. The substrate and Na\(^+\) binding sites on the transporter are formed by TM7, TM8, HP1 and HP2 and so it is possible that substrate and Na\(^+\) binding to the middle of TM7 may transmit conformational changes down to the intracellular edge of TM7 and alter the conformation of K384 and R385. Furthermore, it is possible that the conformational changes induced by glutamate and Na\(^+\) binding (to generate the substrate-activated conductance) may differ from just Na\(^+\) binding alone (to generate the leak conductance). The nature of conformational changes at K384 and R385 may then differentially affect the way that the internal "working part of the transporter" may interact with "outer shell" and D272 (TM5) and D112 (TM2), in particular, to bring about opening of a gate between the outer shell and inner working part of the transporter for anions to pass (Fig. 4). This is clearly a speculative model and more detailed analysis of the roles of the various residues and conformational states of the transmembrane domains is required.

**Should the Substrate-Activated Cl\(^-\) Conductance be Called a Channel or a Leak?**

One of the defining features of ion channels is that ions can permeate through a water filled pore with a typical turnover rate of 6 x 10\(^{6}\) ions per second, which corresponds to a single channel...
conductance of approximately 20pS. Many well defined ion channels have single channel conductances that are much lower than this value and sub pS conductances have been described for a number of channels. However, the estimated single channel conductance for the substrate-activated Cl− conductance of the human glutamate transporter, EAAT1, is approximately 1fS. So, the rate of uncoupled ion movement through the transporter is considerably slower than the typical rate that is observed for many ion channels. This then begs the question as to whether the uncoupled ion movement should be considered an ion channel or a leak? In the following section we will address this question from the perspectives of the functional roles of the Cl− conductance and how the conductance is activated.

In general, the term leak has been used to describe conductances where the molecular basis for its activity are poorly understood, or where the conductance appears to contribute to a background or constitutively active current. So, leak currents contribute to processes such as setting the resting membrane potential or maintaining ion homeostasis. The term leak also has connotations of something going wrong or a poorly controlled mechanism, where as the term channel implies a functional role that can be activated in response to a particular stimulus. So, from this perspective, the question as to whether we should consider the substrate-activated Cl− conductance as a leak or a channel comes down to the question of does this Cl− movement have a functional role and how is it activated? The answer to this question is: yes for some cases, such as EAAT5 in the retina where it clearly plays a physiological role in influencing membrane potential, probably for EAAT4, although this has not been clearly demonstrated; and maybe for others such as EAAT1, EAAT2, EAAT3 (ref. 4) and GLT1, where it may play a role in ion homeostasis (see above for further discussion of these roles). Part of the reason why the Cl− conductance associated with glutamate transporters can influence the cell biology is that under many circumstances, the expression levels of glutamate transporters can be exceedingly high. It has been estimated that the rat equivalent of EAAT2, GLUT-1 is 2% of total brain protein and another way of looking at this expression is that the concentration of transporters in close proximity to the synapse can reach mM. So, although the Cl− conductance of individual transporters may not generate a significant conductance, large numbers of transporters can generate sufficient Cl− flux to play important functional roles. Therefore, under some circumstances the substrate-activated Cl− conductance does play an important functional role and on these grounds, the Cl− conductance is not a process reflecting a faulty transport process, but rather is a functional feature of the transporter that has been retained through evolution to serve a variety of purposes. However, if we turn to the Na+ dependent, but substrate-independent Cl− conductance our view is somewhat different. This conductance generates only 1–5% of the substrate-activated Cl− conductance and will be present under resting conditions and in the absence of any dynamic stimuli, such as glutamate. It does not appear to serve any significant functional role in the transporter, but it may contribute, to some extent, to the resting membrane potential of the cell. Thus, this conductance could reasonably be thought of as arising from a loose seal of the system and as such the term of leak does seem to be appropriate.

Conclusions and Future Directions

The crystal structure of GlpPh, together with a variety of mutagenesis approaches, has provided important insights into how substrates and ions interact with glutamate transporters, but at this stage our understanding of the conformational changes required for transport remains speculative. The structure of the two hairpin loops provides an intriguing possibility that they may form internal and external gates of the transporter. There is some evidence for movements of HP2, but at present there is little information about movements of HP1 that could relate to the gating mechanism. The molecular basis for channel activity is less well understood and there are many details to be worked out. Some of the questions that are still to be adequately addressed include: Where is the gate of the channel? What other regions contribute to the permeation pathway? How does the structure of the permeation pathway change in response to glutamate and Na+ binding? Do the leak and channel conductances utilise the same pore (with slightly different conformations) or does the leak utilise a separate pathway that may overlap with the transport process?

Research on the molecular basis for glutamate transporter functions has undergone a major transformation in the last couple of years and is at a very exciting phase. We anticipate that the future will also provide many conceptual advances, especially in our understanding of how the energy required for transport is harnessed by the protein into providing a very powerful transport process and also the fascinating interplay between transport and channel functions of these proteins.

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