MOLECULAR PHYSIOLOGY

Intimate contact enables transport

Baruch I. Kanner

Sodium-coupled neurotransmitter transporters are essential for neurons to communicate. The high-resolution crystal structure of a bacterial relative hints at how this family of transporters works.

Ion-coupled transporters are essential for all forms of life. These molecular machines carry their cargoes across membranes to help accumulate foodstuffs in cells, maintain cellular pH levels and facilitate communication between nerve cells. Transporters must move their substrates (sugars, amino acids, neurotransmitters, and so on) against sometimes huge concentration gradients. This is accomplished using the energy stored in the electrochemical ion gradients that are maintained across cell membranes. On page 215 of this issue Yamashita *et al.*[1] report the crystal structure of a bacterial leucine transporter, the first structure of a member of the large Na^+/Cl^--dependent neurotransmitter transporter family. It shows that one of the two sodium ions bound in the transporter's binding pocket comes into direct contact with the leucine molecule being carried across the membrane.

Transporters generally 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 the locks in a waterway[2]. 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.

The binding pocket of ion-coupled transporters also has binding sites for the ion that powers the transport process. So the ion and the substrate are transported together — although sometimes in opposite directions — 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 is how the 'driving' ion and the 'driven' substrate move through an ion-coupled transporter.

Yamashita *et al.*[1] studied $LeuT_{Aa}$, a leucine transporter from the bacterium *Aquifex aeolicus*, which is a member of the family of Na^+/Cl^--dependent neurotransmitter transporters[3,4]. Some of the best-known family members function in the central nervous system, where they carry neurotransmitters, the brain chemicals used to signal across the synaptic junctions between neurons. The transporters clear neurotransmitters from the synaptic cleft, terminating the signal and priming the neurons for the next one. Consequently, chemicals that inhibit these proteins — such as cocaine and Prozac — have profound effects on brain function.

Crystal structures of various transporters from other families[5–8] hint that nature has found several solutions to alternating the access of the binding pocket to each side of the membrane. Now Yamashita *et al.*[1] have resolved the structure of $LeuT_{Aa}$ at exceptionally high resolution for a membrane protein (1.65 Å). It 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 (Fig. 1). The sodium ions in the binding pocket are both close to the leucine, with one of them being in direct contact through the carboxyl group (Fig. 1, inset). So it seems that, at least in this transporter and presumably in all the amino-acid-transporting members of this protein family, the coupling between the driving ion and the driven substrate is as direct as it could be.

This direct coupling is an ingenious solution to minimizing leaks where the ion and/or the substrate might permeate through the transporter independently. This mechanism has been proposed previously on the basis of indirect evidence from transporters of other families[9,10], and it may turn out to be used by many transporters. Nevertheless, direct contact may not occur in all transporters; for instance, not all the substrates of transporters related to $LeuT_{Aa}$ have carboxyl groups. In these cases it seems that the carboxyl group is provided by a unique aspartate residue located on transmembrane domain 1 (TM1).

The structure of the functional unit, the $LeuT_{Aa}$ monomer, shows several features known from other transporter structures. One is an 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 twofold axis located in the plane of the membrane. The interface of these repeats forms the binding pocket of the transporter (Fig. 1).

Another feature is the unwinding of

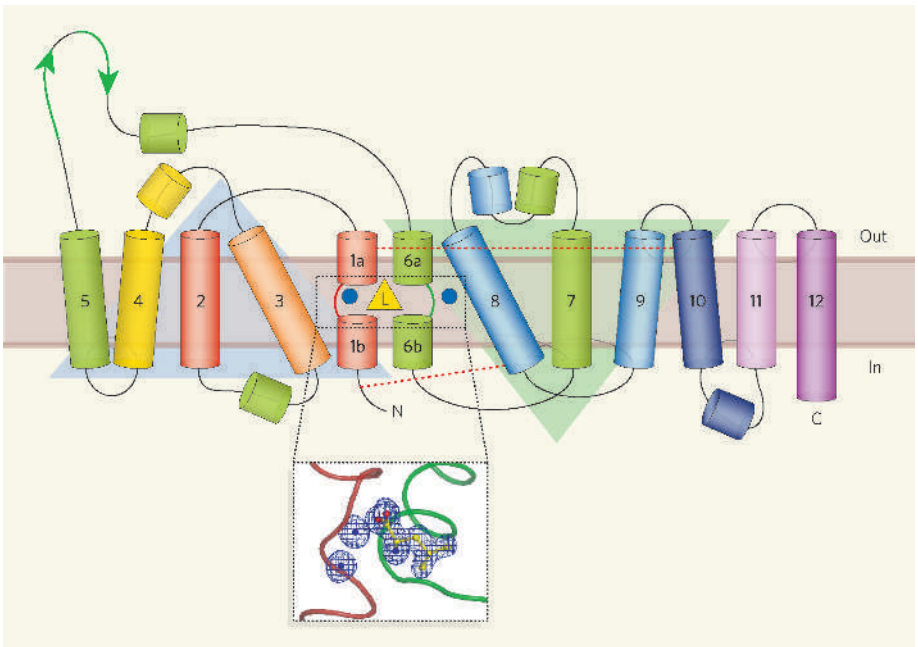

Figure 1 | The $LeuT_{Aa}$ topology revealed by Yamashita and colleagues[1]. Transmembrane domains (TMs) are numbered 1–12, and the oppositely oriented structural repeats encompassing TM1–TM5 and TM6–TM10 are shown as blue and green triangles. TM1 and TM6 are unwound halfway through the membrane, to form the binding pocket for the sodium ions (blue circles) and the leucine cargo (yellow triangle). This structure has its binding sites occluded. 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. The residues involved are close together in three dimensions. Presumably they reciprocally dissociate and associate such that the binding pocket is accessible to the extracellular side when the extracellular pair is dissociated and the intracellular pair is associated, and vice versa. Inset, the binding pocket showing the actual electron densities representing leucine (carbon, yellow; oxygen, red; nitrogen, blue) and the two sodium ions. (Figure adapted from ref. 1.)

membrane-spanning domains, which was first observed in the calcium pump[11]. In $LeuT_{Aa}$, TM1 and TM6 are antiparallel to each other, and have breaks in their helical structure approximately halfway across the membrane (Fig. 1). 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 sodium and leucine. Some of these residues had already been implicated in ion and/or substrate binding by functional studies of mutants of several of the neurotransmitter transporters (cited in ref. 1). Therefore, it appears that the structure reported by Yamashita *et al.* is a physiologically relevant conformation of the transporter.

In this 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 (Fig. 1). The crystal of $LeuT_{Aa}$ also contains a chloride ion (not shown on the figure), but this is not located in the binding pocket. Indeed, in $LeuT_{Aa}$, leucine transport is dependent on sodium but not on chloride[1]. In contrast, in GAT-1, another member of the family, the neurotransmitter GABA is transported together with sodium and chloride ions[12]. So although the overall structure of the neurotransmitter transporters is expected to be similar to that of $LeuT_{Aa}$, there will be variations.

In the future, the $LeuT_{Aa}$ structure will be useful in designing drugs that specifically inhibit the neurotransmitter transporters. Obtaining more 'snapshot' structures representing different transporter conformations will shed light on the most fundamental question: which conformational changes occur during the transport cycle? Or, in terms of the 'lock' model, how is the opening of the external gate coupled to the closing of the internal one, and vice versa? ■

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ENVIRONMENTAL SCIENCE

Carbon unlocked from soils

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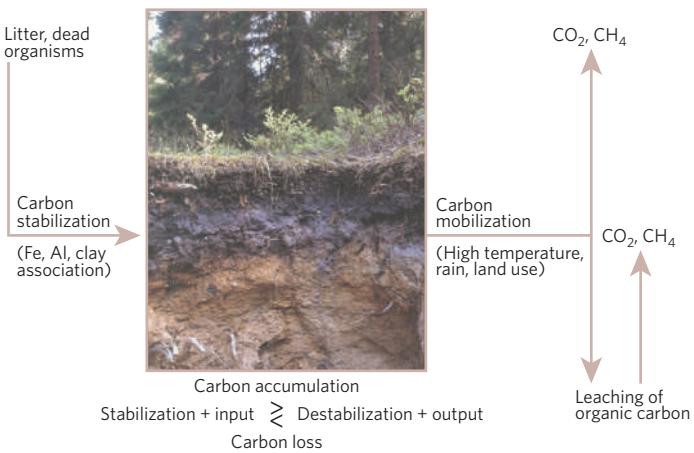
Changes in climate and land use are implicated as the main factors in the large-scale loss of carbon from soils in England and Wales over the past 25 years. The same picture is likely to apply much more broadly.

Soils are major players in the carbon cycle — globally, they store the equivalent of about 300 times the amount of carbon now released annually through the burning of fossil fuels. It is generally assumed that most of the carbon locked up in soils is inert, and stays there. But as Bellamy *et al.* report on page 245 of this issue[1], soil carbon may be more vulnerable to changing climate and patterns of land use than expected.

The carbon involved is known as soil organic carbon (SOC; Box 1). Bellamy *et al.* describe how they have determined the changes in SOC stocks in the top 15 cm of soils in England and Wales during the past 25 years. Their estimates are based on a soil inventory of almost 6,000 sites across all types of land use, resampled on a systematic grid from a pre-existing larger inventory. They find SOC losses of an alarming magnitude. Extrapolating to the entire United Kingdom, Bellamy *et al.* estimate annual losses of 13 million tonnes of carbon. This is equivalent to 8% of the UK emissions of carbon dioxide in 1990, and is as much as the entire UK reduction in CO_2 emissions achieved between 1990 and 2002 (12.7 million tonnes of carbon per year).

These losses thus completely offset the past technological achievements in reducing CO_2 emissions, putting the United Kingdom's success in reducing greenhouse-gas emissions in a different light. Under the Kyoto Protocol, however, countries are not obliged to account for changes in the stock of soil carbon. So an effective climate policy will require a more comprehensive approach that includes all

Box 1|Soil carbon in context



Organic carbon is stored in the top layers of mineral soil as humus or above the mineral soil as peat or litter. This organic material is by no means in equilibrium — neither the carbon concentrations nor the depth of the soil layers are constant, although changes generally occur very slowly. Soils receive dead organic material, known as litter, mainly from the plant cover. This material is decomposed by the soil biota and partly mineralized, and is subsequently released to the atmosphere in the form of carbon dioxide and methane, or by leaching into groundwater[13].

The subtle balance between input and output determines whether a soil is accumulating or losing carbon. Soil organic carbon (SOC) consists of diverse compounds with different chemical and physical properties; for scientific purposes, SOC is divided into an active and a passive pool, the latter being more resilient to further degradation and possibly existing in soil for hundreds to thousands of years. All factors that reduce biological activity, and that stabilize SOC by physical protection or binding to clay silicates or metals, will promote accumulation; factors that increase biological activity and destabilization encourage degradation.

The interplay of these factors is highly complicated. For example, in humid regions, given an adequate supply of moisture, global warming may increase microbial activity and accelerate SOC mobilization; in drier areas, the converse may apply. Changing patterns of land use can also have significant effects. Losses of SOC occur when natural ecosystems are cultivated — because of degradation of soil fertility, intensified soil disturbance and reduced carbon input[10,11]. If conservation measures are applied to degraded soils, however, the SOC content can be maintained or enhanced[14]. **E.D.S. & A.F.**