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50 & 100 YEARS AGO

50 YEARS AGO

It is well known that various organisms can be cooled to very low temperatures in liquid gases without injury, provided that they have previously been desiccated to some extent. Extracellular freezing is considered to be a mode of dehydrating living cells; and some organisms, at least frost-resistant ones, may withstand freezing of this type even at very low temperatures ... Our experiments show that, after sufficient extracellular freezing, an intact insect can be kept alive at an extremely low temperature without any antifreeze agent. We used a 'slug caterpillar', *Cnidocampa flavescens* (Walk.) in the overwintering prepupal stage ... [T]he larvae in their cocoons were first frozen in a special refrigerator, in which the air temperature was lowered from −5° to −90 °C. in 1½ hr. and maintained at that temperature for 45 min. The insects were then rewarmed in air at room temperature. After thawing, about one-third of the sixty larvae revived.

From *Nature* 2 August 1958.

100 YEARS AGO

Mr C. Kenrick Gibbons has presented to the Zoological Gardens a large number of the small fresh-water fish from Barbados known as "millions" (*Girardinus poecilloides*). These little fish, which have been placed in a tank in the tortoise house, are of special interest because of their supposed action in preventing malaria. Malaria is very much less common in Barbados than in other West Indian Islands, and it has been suggested that this freedom is due to the presence of enormous quantities of the "millions" in the fresh-water pools. The little fish are very voracious, and destroy large numbers of the larvae of mosquitoes that spread malaria ... It is understood that experiments are going to be made with the introduction of these fish into tropical countries where malaria is prevalent.

From *Nature* 30 July 1908.

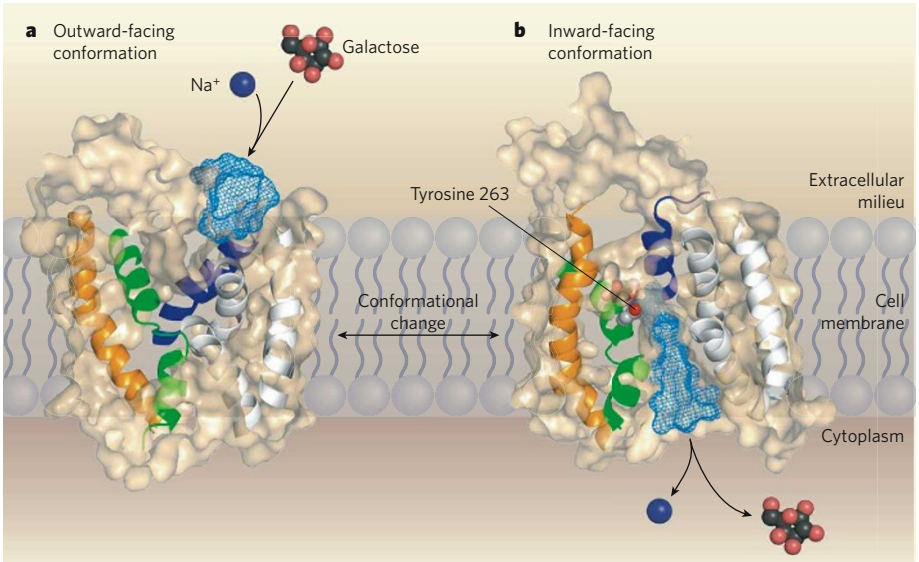

Figure 1 | The entrance and exit routes through the vSGLT transporter. Faham *et al.*[1] find that the core (transmembrane α-helices (TM) 2–11) of the vSGLT structure, which captures this transporter in a conformation facing the inside of the cell, is similar to the core (TM1–TM10) of the LeuT transporter, which faces the outside. A comparison of the two structures allowed the authors to identify the transmembrane helices (orange, green and blue) that undergo structural rearrangements when the protein switches from (**a**) facing outwards, taking up galactose and a sodium ion (Na^+), to (**b**) facing inwards, allowing ion and solute release into the cell. Helices that undergo little movement are shown as white. Tyrosine amino acid 263 is indicated, and extracellular (**a**) and intracellular (**b**) vestibules are shown as a blue mesh. (Adapted from ref. 1.)

as a large hydrophilic vestibule that is separated from the binding pocket by the tyrosine amino-acid residue 263, and the displacement of this residue leads to the release of galactose into the cytoplasm (Fig. 1b). On the other side, the extracellular gate consists of a considerable protein mass that extends from the solute-binding site to the extracellular surface. The opposite situation is encountered in the outward-facing LeuT structure (Fig. 1a and ref. 2), where just a few residues separate the binding pocket and the extracellular vestibule, whereas the intracellular gate is very substantial[2].

How does alternating access work? Clearly, it must involve substrate and cation binding as a trigger for the conformational change[1]. It has been proposed that, during transport, either the two interrupted transmembrane helices themselves[2], or a bundle of these helices plus additional transmembrane domains[5], move as a unit relative to the rest of the protein. The latter model[5] is based on the internal symmetry of the LeuT structure, which is also present in members of other transporter families. In this model, it is proposed that the conformational change leading to the opening of the intracellular-access pathway simultaneously closes its extracellular counterpart. Specifically, it is predicted that the intracellular-access pathway of LeuT is lined by the intracellular parts of TM1, TM5, TM6 and TM8. Studies on another member of the NSS family, SERT, which is the transporter for the neurotransmitter serotonin, reveal[5] that these very transmembrane domains have access to the aqueous intracellular medium, under conditions where the transporter is expected to face the inside of the cell. Such observations not only support this elegant model[5] but are also compatible with the vSGLT structure[1].

A different model[6] is based on the observation that LeuT has a second solute-binding site located at its extracellular vestibule, which was previously shown[7,8] to also accommodate antidepressants. The basic idea here is that the second substrate's binding to the external site results in the opening of the internal gate.

Clearly, additional functional and structural studies are required to shed light on the mechanism of alternating access. For example, after the cargo has been released into the internal compartment, the empty transporter reorients its binding pocket to start a new cycle. Moreover, the mere binding of sodium to the transporter seems to induce conformational changes that enable the transporter to bind the solute. To understand the mechanism of ion-coupled transport, it will be crucial to obtain structural information on the additional conformations of not only transporters of the NSS and SSS families, but also members of other transporter families. ■

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1. Faham, S. *et al. Science* doi:10.1126/science.1160406 (2008).
2. Yamashita, A. *et al. Nature* **437,** 215–223 (2005).
3. Toyoshima, C. *et al. Nature* **405,** 647–650 (2000).
4. Kaback, H. R. *et al. Proc. Natl Acad. Sci. USA* **104,** 491–494 (2007).
5. Forrest, L. R. *et al. Proc. Natl Acad. Sci. USA* **105,** 10338–10343 (2008).
6. Shi, L. *et al. Mol. Cell* **30,** 667–677 (2008).
7. Singh, S. K., Yamashita, A. & Gouaux, E. *Nature* **448,** 952–956 (2007).
8. Zhou, Z. *et al. Science* **317,** 1390–1393 (2007).