50 & 100 YEARS AGO

50 YEARS AGO

'World Population Growth and its Regulation by Natural Means' — Recent discussions on the future of humanity have tended to be rather discouraging, and there are plenty of eminent authorities who are prophesying disaster. World population is now multiplying so fast that at the present rate it is bound eventually to outgrow its food supply, however much that can be increased, and then famine and disease will intervene to prevent further population growth, unless indeed the hydrogen bomb provides an effective and relatively humane alternative to more orthodox Malthusian agents. The only solution seems to be the immediate and universal application of a policy of deliberate family limitation, and few are so optimistic as to believe that this is either practicable or likely to be effective in time to save us from the crash.

From *Nature* 15 September 1956.

100 YEARS AGO

The September issue of the new bi-monthly journal *Concrete* contains admirably illustrated articles on the micro-structure of Portland cement by Dr. C. H. Desch and on reinforced concrete at the Milan Exhibition by Mr. F. R. Farrow. This new addition to the technical periodical literature should prove a valuable source of information to all workers in concrete and cement. The details of the new uses to which concrete and reinforced concrete are put are very remarkable. The use of reinforced concrete as a substitute for timber in exposed positions is rapidly increasing. Railway sleepers, telegraph posts, and fence posts are being tried, and efforts are being made to prove that reinforced concrete is an excellent substitute for brick-work where structures of great height are required.

Also:

We notice with deep regret the announcement that Prof. Ludwig Boltzmann, professor of theoretical physics at the University of Vienna, died by his own hand at Duino a few days ago.

From *Nature* 13 September 1906.

STRUCTURAL BIOLOGY

The ins and outs of drug transport

Shimon Schuldiner

Multidrug transporters provide cells with a defence against toxic chemicals, but they are also responsible for drug resistance. The structures of two such transporters reveal novel aspects of their mechanisms.

Living organisms are constantly assailed by a host of harmful chemicals from the environment. Because of the diversity of these 'xenobiotics', cellular survival mechanisms must deal with an immense variety of molecules. Multidrug transporters (MDTs) supply one such strategy. These ubiquitous membrane proteins recognize a wide array of compounds and remove them from the cell in an energy-dependent manner. As a consequence, the concentration of the offending compound is lowered within the cell, so the compound loses its effectiveness against its cellular target and the cell becomes resistant to it. Such a survival strategy for the individual organism may pose serious problems for the treatment of infectious disease or cancer with drugs.

MDTs have evolved into many different forms to act on a wide range of xenobiotics. The structures of two MDTs[1–3] give an unprecedented view of their mode of action. Murakami *et al.* (page 173 of this issue)[1] and Seeger *et al.* (writing in *Science*)[2] report the structure of AcrB, a transporter that uses the energy of the transmembrane proton gradient. And Dawson and Locher (page 180 of this issue)[3] detail the structure of Sav1866, a transporter that derives its power from the breakdown of ATP molecules. Despite their different structures and energy sources, the transport mechanisms of the two proteins have a common feature: an inward-facing conformation with the substrate-binding site accessible from the cell interior, and an outward-facing conformation with an extrusion pocket exposed to the external medium. The transition between the two conformations is energy dependent.

AcrB is a major MDT from *Escherichia coli* that has many close relatives in other pathogenic bacteria and that confers drug tolerance to these bacteria. AcrB functions in a complex with two accessory proteins — TolC and AcrA — to export a wide variety of substrates. AcrB determines the substrate specificity of the complex, and is also the site where its energy is used. The structures reported by Murakami *et al.*[1] and Seeger *et al.*[2] are consistent with previous structures[4,5] in that they show a threefold

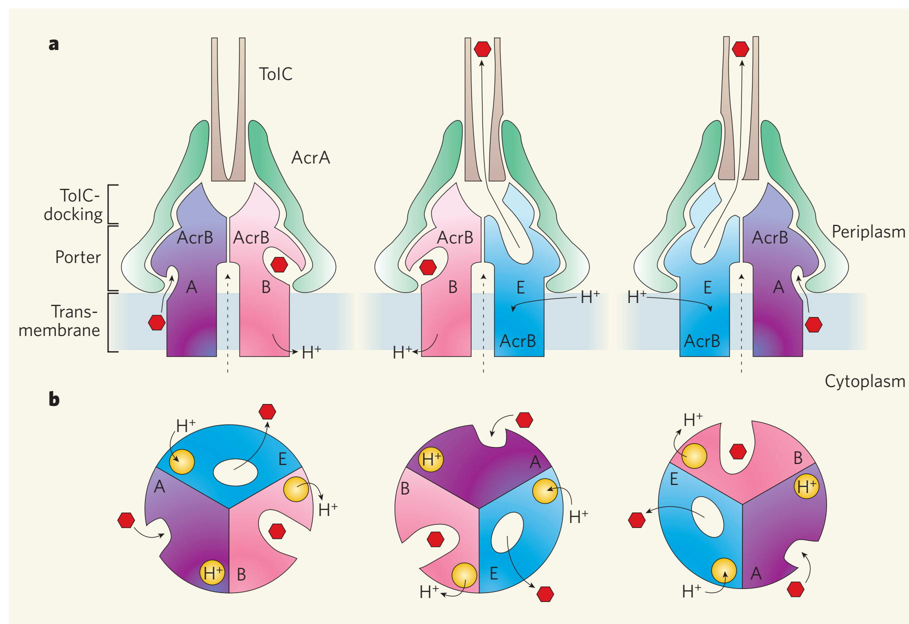

Figure 1 | The structure of the AcrB–drug complex and proposed mechanism of drug transport[1,2]. **a**, The complex is seen from the side, with the drug shown as a red hexagon. The dotted line indicates a possible pathway for substrates moving from the cytoplasm. In this context, Yu *et al.*[5] propose other locations for drug binding sites to the ones shown here. The complex contains three molecules of AcrB, AcrA accessory proteins and the TolC channel to the exterior. The drug is proposed to enter AcrB when it is in the access (A) conformation, before binding more closely to the porter domain of AcrB in the binding (B) conformation. It is then transported to the opposite face and is released from the extrusion (E) conformation of AcrB. Transport of the xenobiotic is powered by the proton (H^+) gradient across the membrane. **b**, The proposed ordered multidrug binding change mechanism of the three-unit AcrB complex. (Adapted from Murakami *et al.*[1] and Seeger *et al.*[2].)

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symmetry with three large subdomains. The functional unit is composed of three identical AcrB units (protomers), each with a transmembrane domain, a so-called porter domain, and a TolC-docking domain (Fig. 1a). The porter domain faces the periplasmic space between the plasma membrane and the outer membrane, where the substrate enters into the substrate-binding pockets. The TolC-docking domain has a central funnel that collects the substrate from each protomer and delivers it to the TolC protein located in the outer membrane.

The new AcrB structures show a noteworthy deviation from symmetry that could not be detected in the previous structures: in the AcrB–drug complex, each of the three protomers has a different conformation. The bound substrate is seen in only one of the three protomers (the 'binding' protomer; Fig. 1b). Exit from one of the vacant binding sites is open towards the TolC funnel, suggesting that this protomer is the form present just after extrusion of the substrate (the 'extrusion' protomer). The other vacant binding site looks to be the state just before substrate binding (the 'access' protomer).

What causes this asymmetry and how it is connected to proton movement (the energy source) remains unclear. However, hints are provided by differences between the three protomers in the transmembrane region where three amino-acid residues were previously suggested to be involved in proton transport. In the 'access' and the 'binding' protomers, a positively charged lysine (Lys 940) is connected by salt bridges to two aspartic acids (Asp 407 and Asp 408). By contrast, in the 'extrusion' protomer, Lys 940 is turned nearly 45° towards a threonine (Thr 978) in the transmembrane region TM11 of the protein, and the salt bridges are abolished. This change may cause the twisting of two transmembrane helices that would influence the movement of other subdomains, including those in the porter domain.

The structure suggests a mechanism whereby drugs enter the binding domain, move through the uptake channel and bind in the large internal pocket. At this point, exit from the binding site is blocked. In the extrusion state, however, the entrance closes and the exit opens. The bound drug is pushed out into the funnel by distortion of the binding pocket. These changes are probably coupled to proton movement across the membrane, and may be induced by protonation and deprotonation of Lys 940, Asp 407 and Asp 408 in the transmembrane domains.

The structure reported by Dawson and Locher[3] is of Sav1866, an MDT from *Staphylococcus aureus*. It is a member of the large 'ABC transporter' family and, as expected from previous studies of this family (for a review see ref. 6), its basic architecture consists of two identical units, with two transmembrane domains that provide a pathway across the membrane, and two cytoplasmic nucleotide-binding domains (NBDs) that break down the ATP nucleotide to provide energy. The structure shows the ATP-

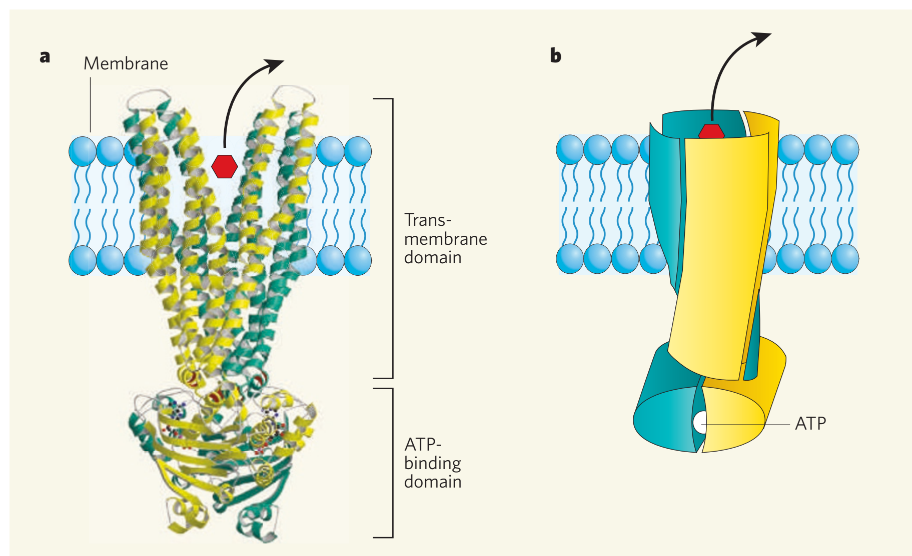

Figure 2 | The structure of the Sav1866 transporter in the ATP-bound conformation[3]. a, Backbone of the Sav1866 transporter, carrying a drug (red), in ribbon representation. The two subunits are shown in yellow and turquoise. **b,** The interaction between the membrane domains and the substrate pathway. (Adapted from Dawson and Locher[3].)

bound state, where the NBDs tightly sandwich the nucleotide between them (Fig. 2a). This is consistent with many kinetic and biochemical studies and with structures of isolated NBD domains containing trapped ATP (for example, from the archaeal ABC protein MJ0796)[7]. It differs, however, from two other ABC-transporter structures: that of the *E. coli* vitamin B12 transporter BtuCD crystallized in the absence of nucleotide[8], and that of the *Staphylococcus typhimurium* transporter called MsbA (refs 9, 10).

Probably the most surprising feature of the new structure[3] is that, in the middle of the membrane, bundles of transmembrane helices diverge into two discrete 'wings' that point away from one another and consist of helices TM1–TM2 from one subunit and TM3–TM6 from the other. So, rather than being aligned side-by-side, the two subunits of Sav1866 are intricately interleaved. Given these constraints, they are unlikely to move independently and their maximum separation during the reaction cycle is therefore limited (Fig. 2b). This view contradicts mechanistic models that suggest that the NBDs join together upon binding ATP and then dissociate upon completing a transport cycle.

In both structures, the power provided by the energy release (either from the proton gradient or ATP) results in long-range modifications in the accessibility of the xenobiotic-binding site(s) that are transmitted by twisting and tilting of one or more transmembrane helices. In the case of Sav1866, the tight association between the two transmembrane domains was unexpected. In the case of AcrB, the surprising asymmetric structure seems to be essential for function and dictates the ordered occupancy of the binding site. This ordered binding mechanism, termed functional rotation by Murakami *et al.*, is similar in principle to the ATP breakdown/synthesis mechanism of the enzyme F_1F_o-ATPase, except that in AcrB there is no mechanical rotation. It is possible that this is a remnant of the evolutionary process that led to the development of true rotary molecular machines.

The AcrB structure also provides a neat explanation of how the cell increases the effectiveness of the system, because one TolC molecule is sufficient to eliminate xenobiotic substrates from three monomers that may be removing three different chemical species. How the substrates are removed from the cytoplasm is not yet clear, however. There is compelling evidence that AcrB eliminates many substrates directly from the periplasmic space before they even enter the cell, but it is very likely that it can also expel xenobiotics that have entered the cytoplasm.

Extensive studies will be needed to understand the transport mechanism fully, but the new structures[1–3] provide some of the ins and outs of the overall process. In addition, the work suggests that a careful re-evaluation of previous structures[4,5,8–10] is called for. The structures may prove to be essential tools in tackling the serious problems posed by drug resistance and the diseases caused by mutations in the human versions of these transporters. ■

Shimon Schuldiner is in the Department of Biological Chemistry, Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel.
e-mail: shimons@cc.huji.ac.il

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