Multi-drug efflux by P-glycoprotein; why has this protein not been stopped yet?

R. Callaghan,[1] R. Ford,[2] M. O'Mara[3] and I.D. Kerr,[4] [1]Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, United Kingdom, [2]Faculty of Life Sciences, Manchester Inter-disciplinary Biocentre, University of Manchester, Manchester M1 7DN, United Kingdom, [3]Molecular Dynamics Group, School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD 4072, Australia and [4]Molecular Dynamics Group, School of Chemistry and Molecular Biosciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom,.

Several ATP-Binding Cassette (ABC) transporters confer resistance to chemotherapy used in the treatment of cancer, bacterial infections and numerous parasitic infections. These proteins confer resistance by preventing the attainment of sufficient intracellular concentrations of cytotoxic drugs through active efflux. This efflux based resistance mechanism is simple, yet highly effective and a widely used strategy. The efflux pumps share the ability to bind, and translocate, a large number of functionally and chemically unrelated drugs. Consequently, the transporters have been collectively grouped as multidrug efflux pumps and share a common structural organisation. Each fully functional efflux pump contains two transmembrane domains (TMD) and two cytosolic nucleotide binding domains (NBD). The TMDs constitute the drug recognition sites and the translocation pathway through the membrane, whilst the NBDs provide energy for translocation (against large concentration gradients) from ATP hydrolysis.

P-glycoprotein (P-gp or ABCB1) is the archetypal multidrug efflux pump from the ABC family and has been established to confer resistance in numerous solid and blood-borne cancers. Given its prevalence and burden to chemotherapy, this protein has been the subject of intensive investigation for over three decades. Its astonishing ability to interact/translocate over 200 compounds has been suggested as a biological enigma. The research in our laboratories has a central objective to provide a molecular mechanism of drug translocation by P-gp. We have utilised biochemical, pharmacological and biophysical approaches to reach this objective and focussed on three specific areas:

How does P-gp bind so many compounds? Convention dictates that substrate binding requires high affinity, directional and selective chemical interactions with the protein. Should this be upheld by P-gp it may require the presence of multiple drug binding sites. Alternatively, the protein may have evolved a binding site capable of mediating the translocation of substrates without the requirement of specific interactions. We have adopted a pharmacological strategy to explore the nature of multi-drug binding by P-gp and two related drug efflux pumps. P-gp does indeed interact with substrates/inhibitors with high affinity and at multiple pharmacologically distinct sites. Moreover, the sites form a complex allosteric communication network.

What is the mechanism coupling drug binding with energy provision? P-gp is able to hydrolyse nucleotide in the absence of any bound substrate and was initially suggested to be an uncoupled transporter. However, the presence of drugs increases the rate of hydrolysis several-fold in a manner suggestive of coupling between domains. Furthermore, there are numerous two-way communication pathways between the drug binding sites and NBDs. We have detailed the involvement and nature of these pathways during the translocation process.

What topographical alterations occur during drug translocation? Structural and biophysical approaches have demonstrated large conformational changes within the TMDs in response to events at the NBDs. It is also clear that TM helices 6 and 12 are intimately involved in propagating this inter-domain coupling. We have demonstrated a number of local topographical changes in TM6/12 and that the two helices mediate their effects in a drug specific manner, indicating multiple communication routes.

Data from these research investigations have been assimilated into a potential translocation mechanism for P-gp, which may form a template for multidrug transport by ABC proteins.