Drug translocation by P-glycoprotein: how do topographical changes in transmembrane helices assist?

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Background: Resistance to chemotherapy is a major causative factor in the frustratingly poor success rate of chemotherapeutic management in cancer. The resistant phenotype is multifactorial involving both cellular and tissue architectural factors. One of the most prevalent, and effective, resistance pathways involves drug efflux pumps from the ATP-Binding Cassette (ABC) superfamily of transport proteins. The efflux proteins confer resistance by ensuring that cancer cells accumulate insufficient concentrations of anti-cancer drugs and thereby evade their cytotoxic actions. P-glycoprotein (a.k.a P-gp or ABCB1) is an archetypal ABC drug efflux pump and is known to confer resistance in a variety of cancer types including colon, liver, breast and a host of haematological disorders. P-gp is known to bind and transport over 200 pharmacological agents; hence it is referred to as a multi-drug transporter. This property remains a biological enigma and remains poorly understood. P-gp comprises four domains, two intramembranous (TMD) and two cytoplasmic (NBD). The TMDs are known to bind drug substrates and provide the translocation pore whilst the NBDs hydrolyse ATP to power the active transport. The TMD and NBDs are linked during translocation to ensure that drug binding and ATP hydrolytic events are efficiently coupled.

Objective: The process of coupling remains undefined, although two transmembrane helices (TM6 & TM12) appear to be intimately involved. As part of an ongoing investigation we aim to provide molecular details on the coupling process and the involvement of these two TM helices. The present investigation describes the role of TM12 in mediating coupling and the conformational changes it undergoes during the translocation process.

Strategy: The investigative strategy involves the mutagenesis based insertion of cysteine residues into various positions along TM12 using the fully functional cysteine-less isoform of P-gp as a template. The single cysteine mutant isoforms were expressed in insect cells with a recombinant baculovirus and purified using affinity chromatography. The purified, reconstituted mutant isoforms were assessed for function to ascertain the functional involvement of the targeted residue positions. In the second phase of investigation, the introduced cysteine residues were measured for their accessibility to covalent modification by a variety of thiol-reactive probes. Using probes with varying biophysical properties, differential labelling would reveal the local environment at each position. Finally, the isoforms were "trapped" in different conformations to assess the changes in local environment and thereby reveal the topography alterations during the translocation process.

Results: This cytosolic region undergoes a shift from a hydrophilic to hydrophobic environment during ATP hydrolysis. Overall the carboxy-proximal region of TM12 appears more responsive to changes in the catalytic state of the protein compared to its amino-proximal region. Thus, the carboxy-proximal region is suggested to be responsive to nucleotide binding and hydrolysis at the NBDs and therefore directly involved in inter-domain communication. These data can be reconciled with an atomic scale model of human ABCB1.

Conclusion: Taken together, these results indicate that TM12 plays a key role in the progression of the ATP hydrolytic cycle in ABCB1, in particular coordinating conformational changes between the NBDs and TMDs.