Pumping ions

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P-Type ATPases are ion pumps which gain their energy directly from the hydrolysis of ATP. This is classified as primary active transport. Active transport in plant cells was for many years one of the main research interests of Professor Alex Hope, one of the founders of the Australian Society for Biophysics, and after whom the McAulay-Hope Prize is in part named. The fact that animals carry out active ion transport seems to have been first recognized by Ernest Overton (1899), but at that time many physical chemists, notably Nernst and Donnan, were searching for equilibrium explanations for the imbalance in ion concentrations across cell membranes. The Donnan effect, in which negatively charged macromolecules in the cell cytoplasm attract cations and repel anions, is able to explain the distribution of K^+ and Cl^- across animal membranes, but not that of Na^+. Na^+ must be actively pumped out of the cytoplasm. The need for a sodium pump was recognized in 1941 by Robert Dean through his work on the storage of blood at low temperatures, and the actual pumping enzyme, the Na^+,K^+-ATPase, was finally isolated in 1957 from crab nerve by Jens Christian Skou (1957), for which he received the 1997 Nobel Prize in Chemistry.

Until recently, progress in resolving the mechanisms of ion pumps has been a long and slow process. Because they undergo relatively large conformational changes as part of their reaction cycles, ion pumps have relatively low turnover numbers. The turnover of the Na^+,K^+-ATPase is approximately 200 s^{-1} at 37˚C. This is far below the measurable ion flux of a single protein of around 5×10^6 s^{-1} (1 pA) using the patch-clamp technique. Electrical recording of single ion pump activity is, thus, currently impossible. In order to measure the kinetics of individual partial reactions, it is necessary to simultaneously activate many pumps. One way of achieving this is *via* an ATP concentration jump, achieved by releasing it photochemically from an inactive precursor ("caged ATP") *via* a laser flash (Kaplan *et al.*, 1978). Unfortunately unphotolysed caged ATP can modify the observed kinetics by blocking ATP binding sites (Clarke *et al.*, 1998). In our research we, therefore, prefer to produce the ATP concentration jump by rapid mixing with ATP *via* the stopped-flow technique. To monitor ion pump kinetics we incorporate an electrochromic fluorescent dye into the membrane adjacent to the protein. The dye RH421 responds to intramembrane electric field strength changes induced by ion binding and release steps of the protein. Using this approach we have been able to answer a number of hitherto unsolved questions regarding the mechanism of the Na^+,K^+-ATPase. For example, we have shown that the enzyme possesses a single ATP binding site which can have both catalytic and allosteric functions in different parts of the enzyme's reaction cycle (Clarke, 2009). We have also shown that in the case of mammalian enzyme, protein-protein interactions occur within the membrane, which cause significant inhibitory changes to the kinetics of the enzyme's partial reactions, but that these interactions are relieved by ATP binding (Clarke, 2009). These findings contribute to the better understanding of the link between the enzyme's quaternary structure and its activity.

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