

Inwardly rectifying Potassium Channels

Olivia Sleator, Steve Prince, *Faculty of Life Science, Manchester University UK*. E-mail: oksleator@hotmail.com

Inwardly rectifying potassium (Kir) channels are integral membrane proteins selective to conducting K^+ ions into cells. These channels are found in many organisms and are involved in a wide range of physiological processes such as propagation of action potential and hormone regulation. The basic topology of Kir channels consist of a tetramer of two transmembrane helices, and a loop containing the selectivity filter. Both the N and C termini of each monomer in the tetramer are intracellular, and these interact to form a large globular domain, which is involved in gating. One of the difficulties in solving the structure of membrane proteins such as Kir channels is obtaining significant quantities of highly purified protein. Rather than relying on the isolation of soluble proteins from natural sources, structural biologists are focused on producing large amounts of target proteins heterologously. Therefore, a number of strategies in this project have been employed to overcome this problem. Murine Kir2.1 has been successfully cloned and overexpressed in the membrane of the methylotrophic yeast *Pichia pastoris*. This yeast was chosen as an expression host as it has many advantages of higher eukaryotic expression systems but it is as easy to manipulate as *E.coli* or *Saccharomyces cerevisiae*. Further investigations into purification and characterisation of the expressed Kir2.1 are currently being undertaken. The intracellular extramembranous domains of the murine Kir2.1 channel have been overexpressed in *E.coli*. This protein forms a stable tetramer and can be purified to a very high level. Crystallisation trials with this pure protein are in progress, as are functional and interaction studies.

Keywords: kir, potassium channels, membrane proteins