9.1.3 Experimental Approaches to Identifying and Understanding Viroporin Function

The simplistic and variable nature of viroporin channel function generally requires the corroboration and correlation between several experimental systems in order to assign such activity confidently to a particular protein. The gold standard for putative viroporins entails the direct link between a requirement during the virus life cycle for alterations in ion homeostasis and the functionality of the protein in question, attested by well-characterised null mutations and/or specific inhibitors with accompanying resistance polymorphisms. This is naturally often a difficult outcome to achieve and is lacking for many proteins which are otherwise generally well accepted as viroporins. Unsurprisingly, the most extensively studied protein, M2, fulfils these criteria due primarily to the relative simplicity of class 1 viroporins, where activity can usually be recapitulated *in vitro* using short peptides corresponding to the TMD.[4] This circumvents issues regarding bacterial expression and is also greatly enabling for structural studies. Nevertheless, despite the considerable amount of work undertaken on M2, additional features of this protein are continually identified, not least of which is the recent discovery of its ability to mediate membrane scission independent of the cellular ESCRT pathway[56] (see Section 9.2.1), plus the way in which inhibitors prevent the flow of protons through M2 channels is still a matter of some debate (see Section 9.2.1.3).

The most immediate issue for the study of viroporins *in vitro* is their hydrophobic nature. Although class 1 proteins are often amenable to the use of peptides representing minimal TMDs, they often remain difficult to synthesise and may behave differently to longer peptides or the full-length protein. However, for the most part, peptides are sufficient to study most aspects of ion channel function and can be very useful in structural studies. For class 2 or 3 viroporins, it is usually the case that the complete protein is necessary for channel activity, necessitating recombinant expression, although some groups have successfully generated examples of full-length, biologically active peptides for HCV p7.[38,39] Circumventing the inherent toxicity associated with bacterial viroporin expression, which itself can form the basis of functional assays (see below), often requires intensive protocols or fusion of the viroporin in question to a partner that prevents its cytopathic effect. This has been successfully pursued in our own laboratory using glutathione-*S*-transferase (GST) as a fusion partner to p7,[37,49] SH[18] and E5,[45] amongst others. Such fusions result in targeting of the protein to bacterial inclusion bodies rather than the inner membrane, significantly reducing toxicity and expediting purification.

Obtaining recombinant protein or peptide permits the demonstration of channel-forming activity using artificial bilayer systems, primarily so-called 'black' lipid membranes.[57,58] Here, highly insulating bilayers separate two buffer chambers containing electrodes that allow manipulation of potential difference. This set-up has been used to analyse many viroporins, including M2,[4] Vpu[26] and p7,[37–39,49] incorporating both small-molecule inhibitors and null mutations. However, these systems can be limited by sensitivity to