available vaccines show poor efficacy in developing countries where the need is greatest. Rotaviruses spread faeco-orally, infecting enterocytes in the small intestine and causing cell death, villus blunting and profuse diarrhoea leading to life-threatening dehydration. A significant hallmark of rotavirus replication is the elevation of cytosolic calcium levels, resulting from increased ER and plasma membrane permeability, which is essential for virus replication and underpins many facets of intestinal disruption. Expression of a single viral non-structural protein, NSP4, is sufficient to recapitulate these effects on calcium homeostasis.[22,310–313]

NSP4 is a 175 amino acid protein that is subdivided into a helical domain at the N-terminus, a coiled-coil region (aa 95–146) for which pentameric crystal structures have been solved,[314–318] and a C-terminal double-layered particle receptor domain, which is essential for its role during the assembly and egress of rotavirus capsids. NSP4 has been found to elevate calcium levels *via* a number of mechanisms, including perturbation of cell signalling,[311] and a secreted C-terminal cleavage product (aa 112–175) induces calcium elevation *via* an endotoxin-like mechanism.[319] However, recent work has attributed direct viroporin activity to the N-terminal region of the protein.[21,23] This region contains multiple helical domains. A short N-terminal helix precedes a TM region that is inserted through an uncleaved signal sequence (H2). This is followed by an extended region (aa 47–92) containing two predicted helical domains, one containing five conserved lysines and the other forming an amphipathic *a*-helix. This region in four sequence-divergent rotavirus subtypes has been shown to mediate membrane permeability in both mammalian and bacterial cells, which is dependent on both the pentalysine motif and the amphipathic character of the second helix, defined by a disruptive mutation.[23] The pentalysine domain also drives insertion of this minimal viroporin domain into membranes, leading to a three TM domain topology being proposed recently for the complete protein.[21] The pentalysine and amphipathic domains are also necessary for cytosolic calcium elevation by full-length protein in mammalian cells and also for induction of membrane permeability, so directly linking direct viroporin activity to this function. Thus, while NSP4 retains multiple activities involving several regions of the protein, it should be feasible to develop small-molecule inhibitors of NSP4 viroporin function that would significantly impair its major role of inducing elevated cytosolic calcium, thereby acting both to inhibit virus replication and to ameliorate diarrhoea symptoms. This could readily be achieved *via* incorporation of minimal viroporin domain peptides into liposome-based screens, potentially incorporating known inhibitors of calcium channels that are already utilised clinically.

9.4 Viroporins Encoded by DNA Viruses

9.4.1 Viroporins of Polyomaviruses

Polyomaviruses are small, non-enveloped dsDNA viruses linked to a number of human diseases such as Merkel cell carcinoma (Merkel cell polyomavirus) and