second helix comprised the TMD. The loop region (aa 33–39) resided at the membrane interface and the more dynamic and flexible C terminal TM region comprised residues 40–56, followed by another short helix (aa 57–63). Opella's group has undertaken extensive solid-state studies of genotype 1b p7 peptides (C27S change incorporated) in detergent micelles, isotropic bicelles and lipid bilayers, which provided further insight into the likely secondary and tertiary structure of p7 monomers.[246,248,252] p7 was shown to possess two TM regions between residues 6–27 and 41–57, each of which consisted of two distinct helical segments. The second and fourth helical segments adopted a tilt relative to the membrane normal of 25° and 10°, respectively. Consequentially, the cytosolic loop in this scenario is extended relative to the solution NMR/molecular dynamics model and a greater proportion of the N- and C-termini extend out with the membrane interface. The search therefore continues for a complete p7 structure with multiple laboratories worldwide focussing on the challenge of both the monomer and the multimeric complex. Given the effects of environment and genotype discussed above, this endeavour may well yield multiple structures along the lines of previous M2 studies.

The gating mechanism of p7 channels is not well defined. Although shown to conduct protons in cellular membranes,[40] *in vitro* experiments have demonstrated that p7 is capable of conducting alkali metal ions, calcium ions and carboxyfluorescein (CF) dye.[37–39,49,61–64,241,247,253] This may be due to the more 'pore-like' qualities of the protein, represent an artefact of individual experimental systems where these are the major ionic species present, or, especially in the case of anionic CF dye, represent an inefficient secondary conductance serving as an indirect measure of channel opening. Nevertheless, proton channel activity was supported by the finding that His17 was essential for genotype 1b p7 activity in liposomes and that increasing pH gradients such that external buffer pH became more acidic led to markedly increased channel activity.[241] However, mutation of His17 in genotype 1a p7 did not abrogate activity *in vitro*[249] and also did not affect virion production by genotype 2a JFH-1 HCV in culture.[233,254] Furthermore, some HCV isolates such as the genotype 2a J6 strain do not contain His residues in the N-terminal helix. Hence genotype-specific differences clearly appear to influence p7 channel activity, making its behaviour seemingly more complex than a standard M2-like model.

Based on available homology and structural information, key positions lining the p7 lumen are likely to comprise ionisable residues such as His17 (Asn in some isolates), Ser21 (Tyr in some) and Tyr31 (His31 in many genotype 2a isolates), which, although located outside the TMD according to solid-state studies,[248] is likely positioned near the mouth of the channel. In addition, several conserved bulky/hydrophobic residues such as Phe25/26 (located at the end of the TMD in solid-state models) and Trp30 could represent gating candidates (see Figure 9.3), supported by a hyperactive channel phenotype for Phe25Ala mutant p7 channels.[48,63] Ionisable residues in particular vary according to HCV genotype, likely affecting channel gating. Interestingly, genotype-dependent differences in acid-mediated p7 channel activation were