Editorial

On 18 September 2003, *FEBS Letters* devoted a Special Issue to the emerging field of Viral Ion Channels. That issue contained 12 papers, which addressed the role of virus-encoded ion channels on the viral life cycle, underscoring their advantage towards an understanding of channel function in terms of protein structure. Four of these papers examined the M2 protein of the influenza virus signifying the impact that this viral ion channel has had in the field. This *FEBS Letters* issue was promptly followed by a workshop held in November 2003 during which a variety of questions related to the M2 proteins of influenza A and B viruses were examined. Pinto and Lamb have summarized below the highlights of the workshop as a 'follow-up' to the Viral Ion Channel Special Issue.

Maurice Montal, Editor

Viral ion channels as models for ion transport and targets for antiviral drug action

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The recent special issue of *FEBS Letters* devoted to Viral Ion Channels (552/1, 18 September 2003) described a number of viral proteins that are thought to have an important role in the viral life cycle by acting as ion channels in their respective viruses. The M2 proteins of influenza A and B viruses are the best studied of the viral ion channels, and recent experimental findings related to these ion channels were reviewed at a workshop held in Evanston, IL, USA on 10 November 2003. Influenza presents a severe threat to public health that is only partially ameliorated by vaccination. Outbreaks of the H5N1 influenza virus in 1997 killed six of the 18 infected individuals, and a highly pathogenic H7N7 avian influenza virus spread to workers in the poultry industry in 2003, killing a veterinarian and causing infections in poultry workers for which there was some evidence of spread from human-to-human. Animal reservoirs serve as a constant source of new variant and reassortant viruses that escape immune surveillance [1], and the M2 protein of influenza A virus is one of the few antiviral drug targets that can be inhibited in order to stifle active influenza infections. It is thus important to study the M2 proteins in order to realize their potential as antiviral targets.

Influenza A and influenza B are related but phylogenetically distinct, membrane-bound viruses that encode the small M2 ion channel proteins [2]. These proton channels are found in the virus membrane and play an essential role in the life cycle of the virus. In fact, the antiviral drug amantadine inhibits replication of the influenza A virus by inhibiting influenza A M2 proton channel activity. Inhibition occurs by preventing acidification of the virus particle while it is contained in the acidic endosome. Acidification of the virion is a prerequisite to viral uncoating; hence, inhibiting the proton channel prevents acidification, stops uncoating and inhibits replication (R. Lamb, Northwestern University). Because the pore-lining residues of the BM2 protein of influenza B virus differ from those of the influenza A M2 protein [3], the antiviral drug amantadine is ineffective against influenza B virus (R. Lamb), but inhibition of the BM2 protein would be expected to be effective in inhibiting replication of influenza B virus, as this virus also requires acidification prior to uncoating [3].

The M2 proteins are attractive models for the study of ion transport across membranes because they are simple molecules that form homo-oligomers in membranes [4,5]; the active state of the influenza A M2 channel is a homotetramer [6]. The formation of tetramers is quite insensitive to mutations at interfacial residues (A. Stouffer, University of Pennsylvania). The pore-lining residues of the influenza A M2 channel have been identified by a combination of cysteine-scanning mutagenesis and inhibition with transition elements [7,8]. These residues are Ala^{30}, Gly^{34}, His^{37}, and Trp^{41}. The His^{37} residue acts as both the pH sensor and selectivity filter and Trp^{41} acts as the activation gate [9] (L. Pinto, Northwestern University). It is probably no coincidence that naturally occurring, amantadine-resistant escape mutations occur at the pore-lining residues Ala^{30} and Gly^{34} [10] but one puzzling escape mutation also occurs at Val^{27}. It was proposed by I. Arkin (Hebrew University, Jerusalem) on the basis of surface plasmon resonance measurements that the channel pore might become widened in escape mutants at this site, permitting amantadine binding to still occur but be ineffective in inhibiting.

Solid-state nuclear magnetic resonance (NMR) measurements of bacterially produced protein (T. Cross and P. Gao, Florida State University and NHMFL, Tallahassee) confirmed earlier predictions based on functional studies and modeling that the transmembrane domain of the influenza A M2 protein forms a helical bundle, each helix of which is oriented at ca. 25° [11] from the perpendicular to the plane of the membrane (although circular dichroism and FTIR measurements indicated a steeper angle; L. Cristian, University of Pennsylvania). Specific labeling of the δ and ε nitrogen atoms

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of His^{37} allowed the protonation state of these atoms to be studied as a function of pH and the pK_a values for the four His^{37} side chains to be determined. The imidazole side chain of histidine in free solution has a pK_a of ca. 6.5, but, as can be expected, the influence of the protein environment within a lipid bilayer was found to influence the pK_a of the His^{37} side chains of the M2 protein. In addition, the presence of a positive charge on one histidine probably influences the pK_a of the remaining uncharged histidines in the tetramer. Two His^{37} residues were found to have pK_a values near 8, a third near 6 and the fourth close to 4. A model of the proton transfer reaction with two titratable sites was presented by J. Lear (University of Pennsylvania). This model was derived from functional studies of conductance vs. pH [12]. Solid-state NMR measurements also indicated the presence of an ordered helical region in the cytoplasmic domain of the influenza A M2 protein that is associated with the inner membrane leaflet [13]. This finding is in agreement with functional findings with truncation mutations and with the resistance to tryptic digestion of residues 44–60 of the cytoplasmic domain [14].

A ready supply of functional protein is essential to all sophisticated structural and biophysical studies. P. Gao reported on a method for the synthesis and refolding of ca. 50 mg quantities of the influenza A M2 monomer in *Escherichia coli* and D. Busath (Brigham Young University) reported on functional reconstitution of this protein in vesicles and bilayers; complete inhibition by amantadine was reported for the former but not the latter preparation. Homotetrameric proteins present an inherent difficulty for interpretation of functional studies because it is not possible to identify which subunit of the tetramer is altered by a particular manipulation. A solution to this problem was presented by G. Kochendoerfer (Gryphon Therapeutics, South San Francisco) who outlined the total chemical synthesis of subunits of the M2 protein [14] and demonstrated how subunits can be assembled on a template to which the ectodomain of each subunit is anchored [15]. This approach would allow just one of the subunits to be modified at a time, in a controlled fashion.

This meeting made it clear that the minimalistic M2 ion channel proteins are a valuable resource for the study of ion transport mechanisms. Uncertainty remains about the role of each of the four transmembrane domain histidine residues and the orientation of the cytoplasmic helices of the influenza A M2 protein relative to the membrane. Great strides in understanding the function of the M2 ion channel protein will result from knowledge of the structure of the protein. Solid-state NMR was revealed to be particularly well-suited to the study of these membrane proteins because this approach directly reports interactions between the relevant ion, the proton, and the atoms of the transporting channel protein. Finally, further knowledge of the structure–function relationship of these two channels will probably allow effective inhibitors to be designed for each of them, providing us with needed antiviral agents that act against influenza virus.

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