Minireview

Demonstrating the intrinsic ion channel activity of virally encoded proteins

Marie L. Kelly[a], James A. Cook[b], Patricia Brown-Augsburger[b], Beverly A. Heinz[b], Michele C. Smith[b], Lawrence H. Pinto[c,*]

[a] *Department of Physiology, Wayne State Medical School, Detroit, MI 48201, USA*
[b] *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[c] *Department of Neurobiology and Physiology, Northwestern University, Hogan Hall 2-140, 2153 N. Campus Drive, Evanston, IL 60208, USA*

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Abstract **This review summarizes the types of evidence that can be invoked in order to demonstrate that a virally encoded protein possesses ion channel activity that is intrinsic to the life cycle of the virus. Ion channel activity has been proposed to be a key step in the life cycle of influenza virus, and the protein responsible for this activity has been proposed to be the M2 protein encoded by the virus. This review contrasts the evidence supporting the conclusion that the A/M2 protein of influenza A virus has intrinsic ion channel activity with the evidence that the 3AB protein encoded by the human rhinovirus possesses intrinsic ion channel activity.**

Key words: Virus; Viral ion channel; Endogenous channel; Influenza virus; Rhinovirus

1. Introduction

If a cellularly encoded protein is suspected to function as an ion channel in a particular type of cell, it is possible to record directly from that cell, perturb the recording conditions, alter the gene expression in that cell type, and make relatively straightforward deductions about the possibility that the protein in question functions as an ion channel or a subunit of an ion channel complex. The situation is more complicated, however, for ion channel proteins that are encoded by viruses because they often encode proteins for which there is no known homologue and because the virally encoded protein might function either in the virus particle (virion) itself or in the infected cell, and, causing further complications for analysis, it might upregulate an endogenous cellular ion channel that is normally silent.

Electrical activity has been reported for several proteins encoded by viruses: NB of influenza B virus [1], VPU of human immunodeficiency virus [2], A/M2 protein of influenza A virus [3], BM2 protein of influenza B virus [4], and K^+ channel protein of Chlorella virus [5], and for some of these proteins the claim has been made that these proteins serve to provide ion channel activity that is needed at some stage during the life cycle of the virus. The aim of this review is to evaluate the type of evidence that can be used to demonstrate intrinsic ion channel activity of a protein, particularly for proteins that are encoded by viruses. In this review we will distinguish an ion channel from a pore by the criteria that ion channels are both selective for a limited range of ions and possess the property of being closed until they are activated by a chemical or electrical stimulus.

The principal experimental obstacle to demonstrating ion channel activity of virally encoded proteins is the difficulty in recording ion channel activity from the extremely small viruses or intracellular organelles that contain the presumed ion channel proteins. Even if it were possible to record from these structures or infected cells it would still be necessary to distinguish the ion channel activity of the virally encoded protein from that of host cell proteins. Instead of recording from a virus, organelle or host cell, the properties of presumed ion channel proteins have been studied in expression systems and these properties compared with those expected from the life cycle of the virus at the relevant stage proposed for the protein. The greater the number of correlations that can be made between the ion channel function predicted from the biological role and the actual ion channel function in an expression system, the stronger is the case that can be made for intrinsic ion channel activity. This review will give two examples of virally encoded proteins, the A/M2 protein of influenza virus and the 3AB protein of rhinovirus, contrasting the evidence that each serves as an ion channel in the life cycle of the virus.

2. The A/M2 protein of influenza virus

The case for intrinsic ion channel activity that plays an essential role in the viral life cycle is perhaps strongest for the A/M2 protein of influenza A virus because this channel has been studied for some time and, it turns out, the antiviral compound amantadine acts by inhibiting the ion channel activity. The influenza A virion is bounded by a membrane formed by budding from the plasma membrane of the infected cell and contains three integral membrane proteins, hemagglutinin (HA), neuraminidase and A/M2 (reviewed in [6]). The evidence that the A/M2 protein has intrinsic ion channel activity does not come only from measurements of its ion chan-

*Corresponding author.
E-mail address: larry-pinto@northwestern.edu (L.H. Pinto).

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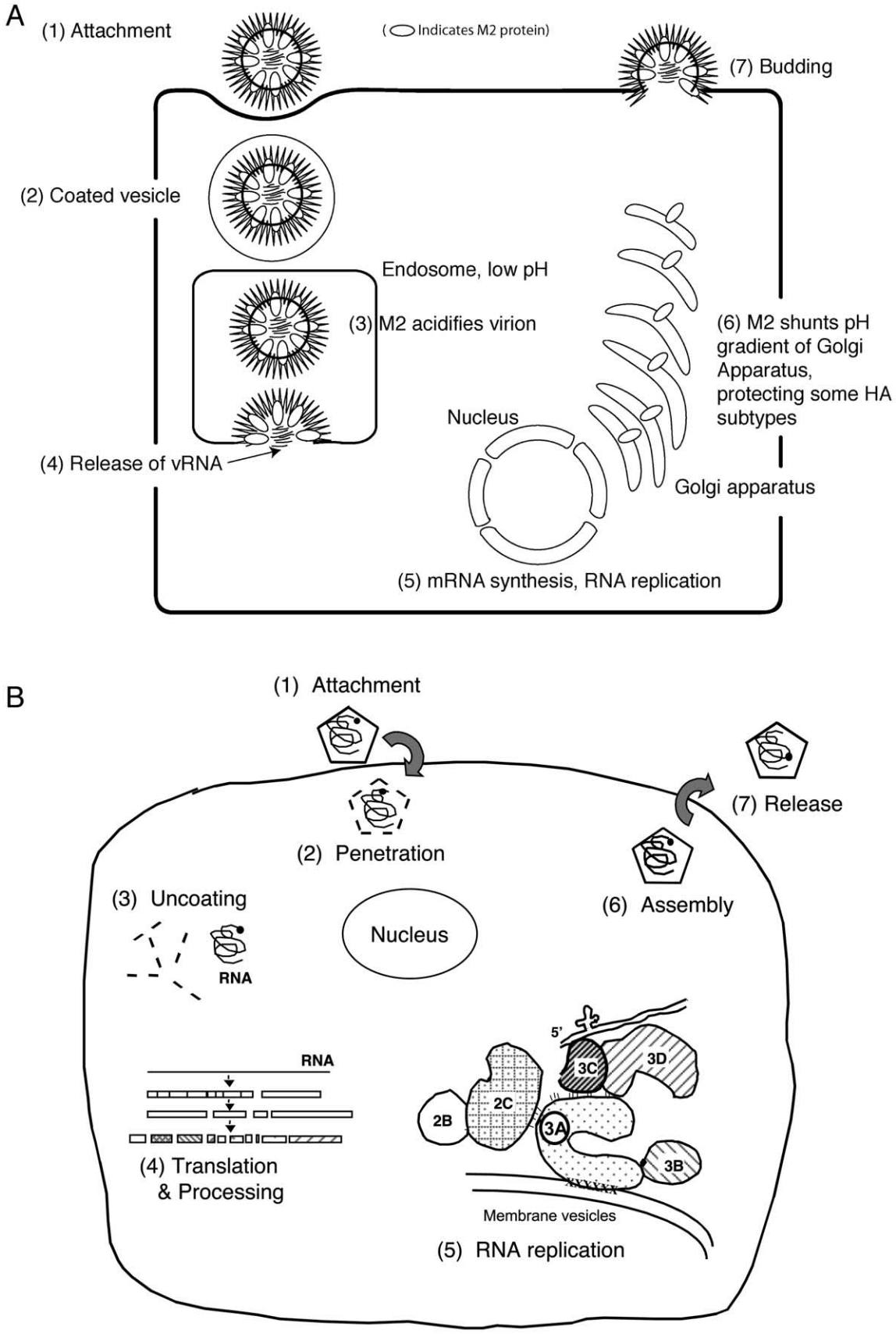

Fig. 1. Comparison of the life cycles of the influenza virus (A) and rhinovirus (B). In A the steps for which the M2 protein is thought to have a role are shown and in B the location of the 3AB protein in the replication complex is shown.

nel activity in expression systems, but also from measurements on mutant A/M2 proteins, a detailed knowledge of the life cycle of the virus, and experiments employing the antiviral drug amantadine (see Fig. 1A).

Influenza A virus HA binds to a sialic acid receptor on the surface membrane of the infected cell and is then endocytosed (reviewed in [6]). While in the acidic endosome, the HA protein undergoes a conformational change to its low pH form that exposes a hydrophobic fusion peptide, and after fusion the interior of the virus is exposed to the cytoplasm of the infected cell. However, fusion alone is not sufficient for the release of the viral genetic material (uncoating) because the ribonuclear proteins (RNPs) are only released from the matrix (M) protein at low pH [7]. For this reason, a low pH step is needed for uncoating. Several lines of evidence implicated the A/M2 protein to be responsible for this acidification and led to the postulate that the A/M2 protein might have ion channel activity [8,9]. This ion channel activity was postulated to cause acidification of the virion while it is contained within the acidic endosome and presumably to continue to provide acidification after the process of fusion has begun in order to maintain the low pH conditions needed to complete release of the RNPs from the M protein (see Fig. 1A, steps 3 and 4).

The first line of evidence implicating the A/M2 protein in the acidification of the virion came from experiments with amantadine, which inhibits the 'early' uncoating step in replication; this evidence has been reviewed [10,11]. Amantadine-resistant 'escape' mutant viruses contain alterations in amino acids in the A/M2 protein transmembrane (TM) domain [12], suggesting that the inhibiting molecule and the A/M2 protein interact in some way. The notion that the A/M2 protein has ion channel activity or is able to accomplish acidification came from experiments in which a 'late' phase of the infective cycle, in which the HA protein is transported from the endoplasmic reticulum to the surface of the infected cells, was studied (Fig. 1A, step 6). It had been known that the HA protein of certain strains of influenza is inserted into the cell membrane in its low pH conformational form when the infected cells were exposed to micromolar concentrations of amantadine [8], presumably due to the low pH of the *trans*-Golgi network [13–15]. When the ionophore monensin, which catalyzes the exchange of Na^+ for H^+ across membranes, was added to the amantadine-treated infected cells, the HA was inserted into the plasma membrane in its high pH form, suggesting that the A/M2 protein and monensin [8,15] both act to shunt the pH gradient across the Golgi membrane.

The A/M2 ion channel protein is a homotetrameric integral membrane protein with each chain of the mature protein containing 96 amino acid residues [9,16–22]. The coding regions for the A/M2 protein have been conserved in all known strains of avian, swine, equine and human influenza A viruses and the amino acid sequence of the A/M2 protein TM domain has been conserved to a greater extent than the remainder of the protein [23]. The TM domain consists of 19 residues and contains both polar residues and a histidine residue, making it possible for the A/M2 protein TM domain to constitute the proteinaceous core (the channel pore) that allows a flux of protons across the membrane.

Direct evidence that the A/M2 protein has ion channel activity was obtained in recordings from oocytes of *Xenopus laevis* [3] and mammalian cells [24–26] that expressed the protein. The oocytes were found to have ion channel activity that was increased when the pH of the solution bathing the N-terminal ectodomain of the protein was lowered [24,27–29]. This activity is so strong that the oocytes and mammalian cells can become acidified in a short time [26,30] when bathed in a mildly acidic solution. The ion channel activity and acidification were both inhibited by amantadine at micromolar concentrations [31]. However, these findings, although consistent with intrinsic ion channel activity, were not sufficient to demonstrate that the activity was intrinsic to the A/M2 protein and not merely the result of upregulation of a normally silent, endogenous protein of the expressing cell. Two additional lines of evidence support this conclusion. First, mutations made in the TM domain of the A/M2 protein produce alterations in the functional characteristics of the protein that are predicted from the biology of the virus infection or anticipated properties of the TM domain. A/M2 proteins with mutations corresponding to those found in amantadine-resistant escape mutant viruses have ion channel activity expressed in oocytes that is resistant to amantadine [27,32]. Second, mutations of two amino acids in the TM domain of the protein produce distinct changes in the proton-handling properties of the recorded activity. Proteins in which His_{37} has been mutated to Gly, Ala or Glu possess ion channel activity different from that of the wild-type (wt) protein in that their activity is not specific for protons and is relatively independent of the pH of the solution bathing the N-terminal ectodomain than the wt protein [28]. A/M2 mutant proteins for which Trp_{41} is mutated to Ala, Cys, Phe or Tyr become activated at higher values of pH than the wt protein and are capable of supporting efflux of protons into external media of high pH, in contrast to the wt protein which 'captures' protons in the cytoplasm [33]. In addition, purified recombinant A/M2 protein, when introduced into artificial lipid bilayers or vesicles, produces ion channel activity that is amantadine-sensitive [34,35] and selective to protons [35]. These results, taken together, show that the A/M2 protein has the necessary ion channel activity in expression systems to explain its postulated role in viral uncoating.

It is important to note that no one piece of evidence would be sufficient to draw the conclusion that A/M2 protein has intrinsic ion channel activity. For example, the activity recorded from artificial lipid bilayers might not reflect the activity found in the virus or the infected cell. The activity recorded in oocytes from wt A/M2 protein might be the result of upregulation of an endogenous ion channel, as is the case for other virally encoded proteins [36]. However, the body of evidence from recordings of ion channel activity and knowledge of the viral life cycle and the proposed role of the protein, together with the results of experiments with the inhibitor amantadine and amantadine-resistant escape mutations, help to make a strong case for the conclusion of intrinsic ion channel activity. Recently, this case for the A/M2 protein has been strengthened by the finding that the B/M2 protein of influenza B virus is an integral membrane protein [4] that has ion channel activity [37].

It should be noted that alterations in the morphological and functional properties of cells ensue with time when they express large quantities of the A/M2 protein. Cells overexpressing the A/M2 protein display a dilation of the lumen of the Golgi apparatus and delay in transport through the secretory pathway that are ameliorated by amantadine and mimicked by treatment with the ionophore monensin [38]. In addition,

we have noticed that cells overexpressing the A/M2 protein also show a greater amount of non-specific 'leakage' current (that is not amantadine-sensitive) when studied with the patch clamp method (mammalian cells) or with two-electrode voltage clamp (oocytes) than do control cells that do not express the protein. The amplitude of these leakage currents often exceeded that of the amantadine-sensitive current after expressing the A/M2 protein for a few days. Thus, secondary effects due to the presence of the A/M2 protein have been found, and these effects are a consequence of the intrinsic activity of the A/M2 protein. These secondary effects give rise to a general increase in 'leakiness' of the expressing cells, and it would be incorrect to conclude that this increase in 'leakiness' in cells expressing the A/M2 protein represents an intrinsic property of the protein.

3. The 3AB protein of human rhinovirus

In contrast to the membrane-bound influenza virus, the human rhinovirus (HRV), a picornavirus, is bounded by an eicosahedral protein capsid. Rather than budding from the plasma membrane, as does the influenza virus, rhinovirus assembly occurs via membrane-associated replication complexes within the infected cell (see Fig. 1B). Replication of human rhinovirus requires the 3AB protein complex which originates from the long polypeptide chain encoded by the viral mRNA. The polypeptide is cleaved by three virally encoded proteases (including 2A and 3C) to produce 11 protein products from three polypeptide regions. The third region, P3, encodes four non-structural proteins (not found in the virion): 3A, 3B, 3C and $3D^{pol}$. A *cis*-acting replication element acts as the primary template for uridylylation by $3D^{pol}$ of VPg (3B), which is covalently linked to all newly synthesized viral positive and negative strand RNAs and to nascent strands of the replication intermediate RNA [39]. VPg (3B) is donated to the replication complex by the precursor protein, 3AB [40], which also interacts with $3D^{pol}$ and most likely tethers $3D^{pol}$ to the replication membrane complex [41]. The 3A region of the 3AB protein contains a conserved hydrophobic region of 22 amino acids [42] and is believed to be involved in sequestering the viral proteins needed for formation and function of the replication complex [43]. Escape mutants from an antiviral benzimidazole drug, enviroxime, map to the 3A region of the 3AB protein [43]. Since this portion of 3AB associates with the membrane and expression of 3AB protein has been shown to modify the permeability of the plasma membrane of cells that express the protein [44,45], we considered the possibility that 3AB protein might possess ion channel activity and that enviroxime might function like amantadine, perhaps inhibiting the ion channel activity.

3.1. 3AB protein and possible ion channel activity

Expression of 3AB protein in *Escherichia coli* causes the bacterial cell to become more permeable to several small molecules [44], and cells infected with the poliovirus have been shown to be more permeable to monovalent cations and small molecules than uninfected cells [46–48]. We attempted to test for possible ion channel activity of the 3AB protein by expressing the protein in oocytes of *X. laevis*, but we found that after injection of mRNA encoding the 3AB protein an upregulation of an endogenous, cellular protein occurred; this type of upregulation has been found for other virally encoded proteins as well [49]. For this reason, we used a different approach to test for ion channel activity associated with the 3AB protein. This approach is to purify the protein, incorporate it into planar lipid bilayers, and test these bilayers for ion channel activity.

Vesicles containing 3AB protein were incorporated into lipid bilayers using the nystatin–ergosterol technique, which allows the quantification of the number of vesicles that were incorporated into the bilayer prior to the appearance of ion channel activity (see [50]). Briefly, purified 3AB protein, 1 μg/μl, was incorporated into artificial vesicles using the freeze/thaw/sonicate technique. Vesicles were composed of phosphatidylethanolamine:phosphatidylcholine:phosphatidylserine:ergosterol in the weight ratio 2.1:0.9:1:1 with 0.05 μg/μl of

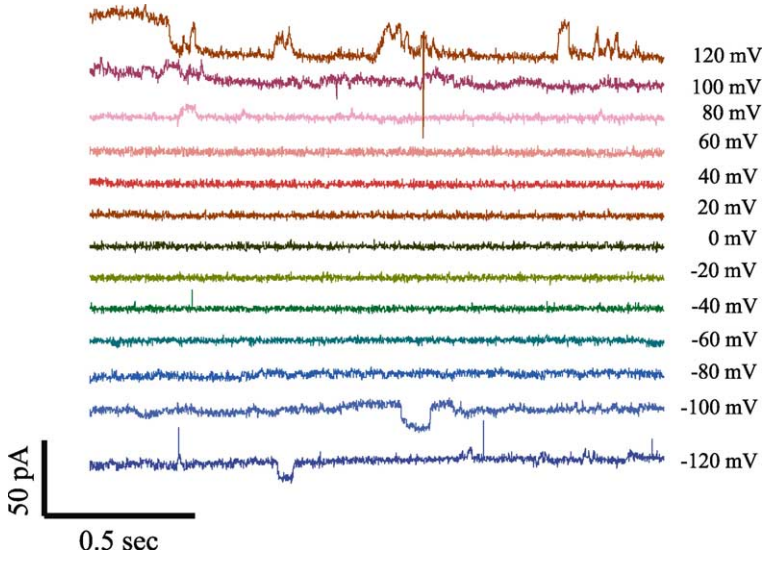

Fig. 2. Example of variable activity of the 3AB protein from HRV-14 introduced into a planar lipid bilayer using the nystatin–ergosterol technique. Traces are offset by 20 pA each. The channel-like activity remains primarily closed at negative voltages and at positive voltages less than +60 mV. Bath solutions for asymmetric 3AB analysis were 428 or 645 mM NaCl, 8 mM HEPES *cis* and 150 mM NaCl, 8 mM HEPES *trans*.

nystatin. These vesicles were fused with a 200–400 μm diameter planar lipid bilayer.

Electrical activity was observed in the form of multiple conductance states for which the current was carried fairly non-selectively by cations. An example of three main conductance states; a medium amplitude (80 pS ± 1 pS S.E.M.; $n = 6$) conductance, a slow gating subconductance (29 pS ± 5 pS S.E.M.; $n = 6$) state and a full conductance (103 pS ± 8 pS S.E.M.; $n = 6$) state characterized by rapid gating were observed [51]. In addition to these three conductance states, a sporadically occurring state of large conductance (as large as 1 nS) and medium conductance states which were highly voltage-dependent when studied in asymmetric monovalent salt solutions were also observed (Fig. 2) [51]. This electrical activity was greatly reduced by boiling the protein prior to incorporation into lipid vesicles. However, none of the electrical activity was significantly inhibited by the antiviral enviroxime.

In order to determine if the electrical activity observed in bilayers was enough to cause the membrane to become permeable to large molecules, 3AB protein was incorporated into vesicles containing fluorescently labeled dextrans (FITC-dextran) of various sizes. The efflux of the dextrans from these vesicles was quantified. We found that FITC-dextran of molecular weight as high as 70 000 Da was able to escape from 3AB-containing vesicles but not from control vesicles (Fig. 3). The results of this experiment are consistent with the large (1 nS) conducting states seen for the ion channel activity associated with the 3AB protein. However, the efflux was also not significantly inhibited by enviroxime.

Ion channel activity of crude replication complexes. Because the 3AB protein is found in the replication complex in virus-infected cells [52], we tested HRV-14 replication complexes for ion channel activity. To do this, we incorporated crude replication complexes into planar lipid bilayers. We found that the replication complex also demonstrated ion channel activity when incorporated into planar lipid bilayers (Fig. 4). The ion channel activity of the replication complex was similar to that of 3AB protein in two respects. First, both channels were fairly non-selective to various ions with a preference of Na > K. Second, both demonstrated mild pH sensitivity, with both showing a decrease in conductance as pH was decreased. The channels also differed in several ways. The ion channel activity associated with the replication complex had a longer open time with a larger primary conductance state of 313 pS (± 10 pS S.E.M.; $n = 3$) than that associated with the 3AB protein [51]. The ion channel activity associated with the 3AB protein also had more conductance states than did that of the replication complex.

3.2. 3AB channel or 3AB pore?

The above results demonstrated that the 3AB protein is capable of electrical activity in bilayers and permits leakage of dextrans from vesicles. The question remained whether the protein acted as an ion channel or as a pore. To help decide this question we determined the quantity of 3AB protein that was associated with a bilayer that had electrical activity. This was done by counting the number of vesicles that were incorporated in the membrane before 3AB electrical activity was observed and then multiplying the number of vesicles by the average 3AB protein content per vesicle to obtain the number of 3AB molecules that were incorporated in the bilayer.

Vesicle incorporation was determined by counting the num-

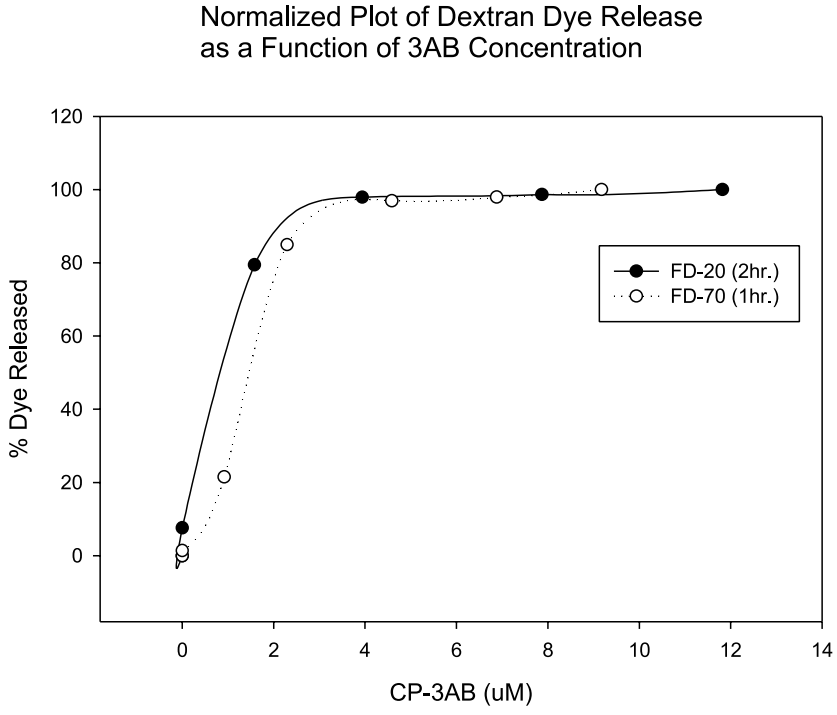

Fig. 3. Release of entrapped FITC-dextran from artificial vesicles into which 3AB protein (His-tagged, S-sulfonate form) had been incorporated in various concentrations. Vesicles containing fluorescein dextrans were prepared with a weight ratio of 9.1:1 of phosphatidylcholine to phosphatidylglycerol. The dried lipid film on the bottom of a round bottom flask was resuspended in phosphate-buffered saline (PBS) containing 10–20 mg/ml of FITC-dextran by vortexing, followed by sonication and freeze-thawing to produce large unilamellar vesicles. The vesicles were sized through a 0.1 μm filter and then washed four times with PBS. 3AB protein was incorporated into the vesicles at each concentration and the release of the dye into the supernatant was measured after the times indicated for each concentration by high performance size exclusion chromatography using a TSK-3000SW column equilibrated with PBS (pH 7.4, 1 ml/min excitation 496 nm, emission 518 nm).

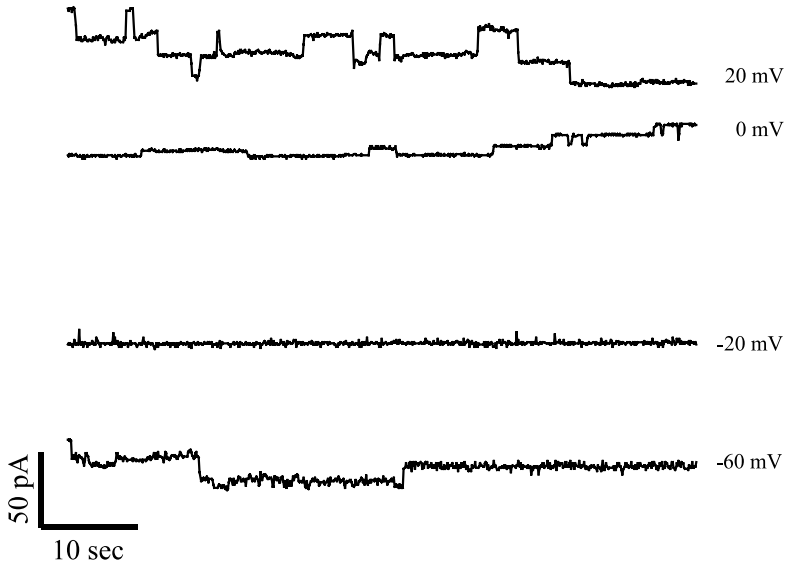

Fig. 4. Activity of crude replication complex from HRV-14 introduced into a planar lipid bilayer using the nystatin–ergosterol technique. Three replication complex (RC) channel-like activities are demonstrated at 0 mV and +20 mV. Notice the difference in open times between 3AB (Fig. 2) and RC. NaCl concentrations used for replication complex analysis were identical to those for 3AB analysis but were done in diethylpyrocarbonate-treated water.

ber of instances of transient currents induced by nystatin–ergosterol ('fusion spikes') that appeared across the bilayer. These currents are transient because the ergosterol, which is necessary for nystatin channel formation, diffuses into the lipids in an ergosterol-free bilayer once the vesicle incorporates.

Amino acid analysis was used in conjunction with analysis of fusion spikes to compute the number of 3AB protein molecules per channel electrical activity observed. This was done by measuring the amount of 3AB protein incorporated into vesicles from a known concentration of the protein. We found that the average number of 3AB protein molecules per vesicle depended on the pH at which the vesicles were formed. At pH 7.2, we calculated that the average number of 3AB molecules per channel activity was 323 ± 14. We observed that an average of four fusion events occurred before ion channel activity was observed. The fraction of molecules that actually participated in the ion channel-like activity we observed is not known, but the average number of molecules per activity is too large to make a convincing case that the electrical activity was the result of an ion channel [53–55].

A comparison of the evidence that the 3AB protein and the A/M2 protein have intrinsic ion channel activity is informative. The biological evidence indicating a need for an ion channel to provide acidification of the influenza virion is strong, but the biological evidence indicating that an ion channel activity was needed during the rhinovirus life cycle is not as compelling. The evidence for the A/M2 protein included the need for acidification activity for viral uncoating and association of the A/M2 protein with acidification activity. On the other hand, the suspicion that the 3AB protein might be an ion channel was based only on the hunch that an ion channel or pore was needed in the replication complex during the assembly phase of the life cycle. The biophysical evidence that the A/M2 protein has intrinsic ion channel activity is supported by numerous findings: experiments in which the activity was recorded in various types of cells, the correlation of the sensitivity of the ion channel activity of mutant proteins to amantadine with the amantadine sensitivity of the virus expressing these mutant proteins, and the finding of ion channel activity from purified A/M2 protein. In contrast, the biophysical evidence that the 3AB protein forms an ion channel during replication is weak. First, its possible ion channel activity cannot be studied in one of the most useful expression systems, the *Xenopus* oocyte, because upregulation of an endogenous oocyte channel activity follows injection of mRNA encoding the 3AB protein. Second, electrical channel activity measured in bilayers requires the insertion of several hundred 3AB molecules (although this figure is an upper limit), and this activity is not significantly inhibited by enviroxime. Third, the increased permeability of the plasma membrane of cells that express the 3AB protein might reflect the same phenomenon that affects cells which have expressed the A/M2 protein for some time: the condition of the cell might be affected by the viral protein expression in such a fashion as to make the permeability increase. Release of the rhinovirus virion from the infected cell is essential to replication, but this step could be accomplished either by cell lysis or with a lipidic pore that would not need the ionic specificity of the A/M2 channel.

4. Conclusion

Comparing the evidence that these two proteins possess intrinsic ion channel activity shows that the demonstration of intrinsic activity requires the convergence of many lines of evidence coming from the biology of the virus and biophysical measurements. No one piece of evidence alone is sufficient to make a convincing case.

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References

[1] Sunstrom, N.A., Premkumar, L.S., Premkumar, A., Ewart, G., Cox, G.B. and Gage, P.W. (1996) J. Membr. Biol. 150, 127–132.
[2] Schubert, U., Ferrer-Montiel, A.V., Oblatt-Montal, M., Henklein, P., Strebel, K. and Montal, M. (1996) FEBS Lett. 398, 12–18.
[3] Pinto, L.H., Holsinger, L.J. and Lamb, R.A. (1992) Cell 69, 517–528.
[4] Paterson, R.G., Takeda, M., Ohigashi, Y., Pinto, L.H. and Lamb, R.A. (2003) Virology 306, 7–17.
[5] Plugge, B., Gazzarrini, S., Nelson, M., Cerana, R., Van Etten, J.L., Derst, C., DiFrancesco, D., Moroni, A. and Thiel, G. (2000) Science 287, 1641–1644.
[6] Lamb, R.A. and Krug, R.M. (2001) in: Fields Virology (Knipe, D.M. and Howley, P.M., Eds.), pp. 1487–1531, Lippincott, Williams and Wilkins, Philadelphia, PA.
[7] Zhirnov, O.P. (1992) Virology 186, 324–330.
[8] Sugrue, R.J., Bahadur, G., Zambon, M.C., Hall-Smith, M., Douglas, A.R. and Hay, A.J. (1990) EMBO J. 9, 3469–3476.
[9] Sugrue, R.J. and Hay, A.J. (1991) Virology 180, 617–624.
[10] Hay, A.J. (1992) Semin. Virol. 3, 21–30.
[11] Lamb, R.A., Holsinger, L.J. and Pinto, L.H. (1994) in: Receptor-Mediated Virus Entry into Cells (Wimmer, E., Ed.), pp. 303–321, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[12] Hay, A.J., Wolstenholme, A.J., Skehel, J.J. and Smith, M.H. (1985) EMBO J. 4, 3021–3024.
[13] Ciampor, F., Thompson, C.A., Grambas, S. and Hay, A.J. (1992) Virus Res. 22, 247–258.
[14] Grambas, S., Bennett, M.S. and Hay, A.J. (1992) Virology 191, 541–549.
[15] Grambas, S. and Hay, A.J. (1992) Virology 190, 11–18.
[16] Lamb, R.A., Zebedee, S.L. and Richardson, C.D. (1985) Cell 40, 627–633.
[17] Zebedee, S.L., Richardson, C.D. and Lamb, R.A. (1985) J. Virol. 56, 502–511.
[18] Lamb, R.A. and Choppin, P.W. (1981) Virology 112, 729–737.
[19] Holsinger, L.J. and Lamb, R.A. (1991) Virology 183, 32–43.
[20] Panayotov, P.P. and Schlesinger, R.W. (1992) Virology 186, 352–355.
[21] Sakaguchi, T., Tu, Q., Pinto, L.H. and Lamb, R.A. (1997) Proc. Natl. Acad. Sci. USA 94, 5000–5005.
[22] Tobler, K., Kelly, M.L., Pinto, L.H. and Lamb, R.A. (1999) J. Virol. 73, 9695–9701.
[23] Ito, T., Gorman, O.T., Kawaoka, Y., Bean Jr., W.J. and Webster, R.G. (1991) J. Virol. 65, 5491–5498.
[24] Chizhmakov, I.V., Geraghty, F.M., Ogden, D.C., Hayhurst, A., Antoniou, M. and Hay, A.J. (1996) J. Physiol. 494, 329–336.
[25] Wang, C., Lamb, R.A. and Pinto, L.H. (1994) Virology 205, 133–140.
[26] Mould, J.A., Drury, J.E., Frings, S.M., Kaupp, U.B., Pekosz, A., Lamb, R.A. and Pinto, L.H. (2000) J. Biol. Chem. 275, 31038–31050.
[27] Pinto, L.H., Dieckmann, G.R., Gandhi, C.S., Shaughnessy, M.A., Papworth, C.G., Braman, J., Lear, J.D., Lamb, R.A. and DeGrado, W.F. (1997) Proc. Natl. Acad. Sci. USA 94, 11301–11306.
[28] Wang, C., Lamb, R.A. and Pinto, L.H. (1995) Biophys. J. 69, 1363–1371.
[29] Mould, J.A., Li, H.-C., Dudlak, C.S., Lear, J.D., Pekosz, A., Lamb, R.A. and Pinto, L.H. (2000) J. Biol. Chem. 275, 8592–8599.
[30] Shimbo, K., Brassard, D.L., Lamb, R.A. and Pinto, L.H. (1996) Biophys. J. 70, 1336–1346.
[31] Wang, C., Takeuchi, K., Pinto, L.H. and Lamb, R.A. (1993) J. Virol. 67, 5585–5594.
[32] Holsinger, L.J., Nichani, D., Pinto, L.H. and Lamb, R.A. (1994) J. Virol. 68, 1551–1563.
[33] Tang, Y., Zaitseva, F., Lamb, R.A. and Pinto, L.H. (2002) J. Biol. Chem. 277, 39880–39886.
[34] Tosteson, M.T., Pinto, L.H., Holsinger, L.J. and Lamb, R.A. (1994) J. Membr. Biol. 142, 117–126.
[35] Lin, T.I. and Schroeder, C. (2001) J. Virol. 75, 3647–3656.
[36] Shimbo, K., Brassard, D.L., Lamb, R.A. and Pinto, L.H. (1996) Biophys. J. 70, 1335–1346.
[37] Mould, J.A., Paterson, R.G., Takeda, M., Ohigashi, Y., Venkataraman, P., Lamb, R.A. and Pinto, L.H. (2003) Dev. Cell 5, 175–184.
[38] Sakaguchi, T., Leser, G.P. and Lamb, R.A. (1996) J. Cell Biol. 133, 733–747.
[39] Yang, Y., Rijnbrand, R., McKnight, K.L., Wimmer, E., Paul, A., Martin, A. and Lemon, S.M. (2002) J. Virol. 76, 7485–7494.
[40] Porter, A.G. (1993) J. Virol. 67, 6917–6921.
[41] Hope, D.A., Diamond, S.E. and Kirkegaard, K. (1997) J. Virol. 71, 9490–9498.
[42] Towner, J.S., Ho, T.V. and Semler, B.L. (1996) J. Biol. Chem. 271, 26810–26818.
[43] Heinz, B.A. and Vance, L.M. (1995) J. Virol. 69, 4189–4197.
[44] Lama, J. and Carrasco, L. (1992) J. Biol. Chem. 267, 15932–15937.
[45] Lama, J. and Carrasco, L. (1995) FEBS Lett. 367, 5–11.
[46] Lama, J. and Carrasco, L. (1996) J. Gen. Virol. 77, 2109–2119.
[47] Lopez-Rivas, A., Castrillo, J.L. and Carrasco, L. (1987) J. Gen. Virol. 68, 335–342.
[48] Munoz, A. and Carrasco, L. (1983) Eur. J. Biochem. 137, 623–629.
[49] Shimbo, K., Brassard, D.L., Lamb, R.A. and Pinto, L.H. (1995) Biophys. J. 69, 1819–1829.
[50] Kelly, M.L. and Woodbury, D.J. (1996) Biophys. J. 70, 2593–2599.
[51] Kelly, M.L. and Woodbury, D.J. (2003) in: Planar Lipid Bilayers and their Applications (Tien, H.T. and Ottova, O., Eds.), pp. 699–721, Elsevier, Amsterdam.
[52] Doedens, J.R. and Kirkegaard, K. (1995) EMBO J. 14, 894–907.
[53] Chernomordik, L., Chanturiya, A.N., Suss-Toby, E., Nora, E. and Zimmerberg, J. (1994) J. Virol. 68, 7115–7123.
[54] Lear, J.D., Wasserman, Z.R. and DeGrado, W.F. (1988) Science 240, 1179–1181.
[55] Ehrlich, B.E. (1992) in: The Heart and Cardiovascular System (Fozzard, H.A., Ed.), pp. 551–560, Raven Press, New York.