

Minireview

Entry of animal viruses and macromolecules into cells

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Abstract

The entry of animal viruses into cells is mediated by conformational changes in certain virion-particle components. These changes are triggered by the binding of virions to receptors and are influenced by low pH during receptor-mediated endocytosis. These conformational alterations promote the interaction of some viral proteins with cellular membranes thereby leading to transient pore formation and the disruption of ionic and pH gradients. The entry of toxins that do not possess receptors on the cell surface is promoted during the translocation of the virus genome or the nucleocapsid to the cytoplasm. A model is now presented which indicates that efficient virus translocation through cellular membranes requires energy, that may be generated by a protonmotive force. The entry of some animal viruses, as promoted by low pH, should thus only take place when a pH gradient and/or a membrane potential exist, but will not take place if these are dissipated, even if virion particles are present in an acidic environment.

Key words: Animal virus entry; Protonmotive force; Endosome acidification; Virus receptor

1. Introduction

For animal viruses to infect cells, translocation of the genome to the cell interior is required. Current models describing virus entry are based on mechanical interactions of virion proteins with membranes and no energy requirements are envisaged. For viruses such as poliovirus or adenovirus, that are devoid of a lipid envelope, suggested mechanisms for genome entry involve pore formation in membranes, through which the genome passes [1]. A further possibility is that the endosomal membrane is disrupted [1–3], with the consequent pouring of the endosomal contents to the cytoplasm. Viruses possessing a lipid membrane that surrounds the nucleocapsid contain glycoproteins involved in receptor recognition and virus entry into cells [4,5]. These viral glycoproteins change their conformation, as mediated by receptor binding or acidic pH, thereby triggering fusion between the virion and cellular membranes [4,5]. Viruses, such as Sendai virus, are able to fuse their lipid envelope directly with the plasma membrane in a pH-independent manner, whereas other viruses, such as Semliki Forest virus, fuse their envelope with the endosomal membrane upon acidification [6]. Under all circumstances, however, fusion is promoted by specialized viral glycoproteins whose activity is triggered by conformational changes within the glycoprotein [7]. Two major factors can contribute to these changes: (i) binding of the virus particle to the receptor, and/or (ii) low pH [6,8]. The exact contribution that each of these factors has in the induction of conformational changes in virion components during virus entry remains to be determined [9,10].

The fact that a low-pH step is required for the entry of some viruses into cells has been taken as evidence that an acidic pH is necessary for the induction of conformational changes in the viral fusion glycoprotein [7] and no energy requirement has been implicated, provided that fusion takes place [5]. Nevertheless, this simple model does not explain several experimental findings including, for example, the efficient translocation of other macromolecules into cells by virus particles [11,12].

2. Virus particles promote the entry of other macromolecules into cells

Viral particles efficiently permeabilize cells to protein toxins that, because of the absence of suitable receptors, are otherwise unable to cross the membrane [11] (see Fig. 1). The toxins are efficiently delivered to the cytoplasm shortly after addition of virus to the medium, and almost 100% of the cells become permeabilized within a few minutes [11,13,14]. All animal viruses tested, including Semliki Forest virus, vesicular stomatitis virus, vaccinia virus, adenovirus and poliovirus, induced this phenomenon [2,11,13–15]. These findings suggest that the viral particle contains a component that not only promotes the entry of the viral nucleocapsid into the cell, but also translocates other macromolecules, that are not physically bound to the particles, across the cellular membrane to the cytoplasm. Permeabilization of human cells by poliovirus to protein toxins during virus entry requires the uncoating of virus particles [16] and the functioning of the vacuolar proton-ATPase [17–19] (see Fig. 1). The use of macrolide antibiotics, such as bafilomycin A1 and concanamycin A, that are selective and

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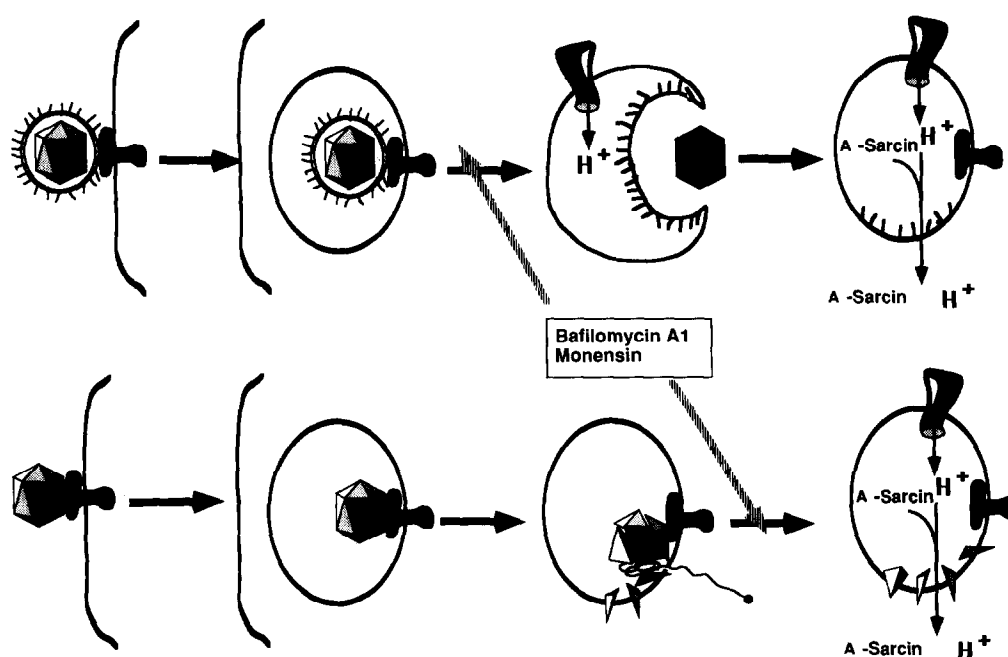


Fig. 1. Mechanism of virus entry through receptor-mediated endocytosis. The co-entry of other macromolecules, such as alpha-sarcosine, is also shown. The blockade of the vacuolar proton-ATPase by bafilomycin A, or the dissipation of the proton gradient by monensin, inhibits the entry of Semliki Forest virus (upper panel), but not poliovirus (lower panel). In both cases the co-entry of alpha-sarcosine requires an active vacuolar proton-ATPase.

powerful inhibitors of the vacuolar proton-ATPase is crucial to elucidate the molecular mechanisms of virus entry and the early membrane permeabilization. Fig. 2 summarizes the results obtained on the inhibition of virus entry and alpha-sarcosine co-entry by bafilomycin A1. The use of this simple test gives clues about the route followed by a given animal virus to infect cells.

Permeabilization by adenovirus particles has also been used to introduce DNA into cells, by employing a receptor-mediated gene delivery system. Plasmids can bind to cells when they are complexed with transferrin-polylysine molecules [20,21]. The entry of these complexes is enhanced when adenovirus particles are present in the culture medium. Even the conjugation of influenza fusogenic peptides (as derived from the hemagglutinin molecule) with the DNA-complexes, enhances gene delivery [22]. Such a synthetic system is similar to a virus particle in that nucleic acids are coated with proteins that interact with them; these proteins contain a moiety that binds the complex to receptors. Nevertheless, although this complex is able to bind to the cell surface, it still lacks the permeabilizing capacity of virus particles. This property can be provided by the addition of virion particles to the system.

3. Virus entry opens transient pores in the membrane

Virions modify their conformation upon receptor binding thereby allowing the insertion of virion compo-

nents into the cellular membrane [23,24]. An acidic pH might also influence these conformational changes [5], although such additional alterations may not be strictly required for fusion, since enveloped animal viruses can fuse at neutral pH [25] and infect cells that are deficient in endosome acidification [9]. It was observed initially by Klemperer [26] that absorption of viruses to cells induces changes in membrane permeability [12,27]. Such changes cause the diffusion of cations and protons thereby resulting in a drop in membrane potential [12,27,28]. Indeed, once viral proteins are inserted into the membrane they are able to open pores, through which protons and other ions can pass if the gradient is favorable. Virus particles appear to contain structures identical to ion channels [29]. Upon insertion in cellular membranes, these structures can open pores [30–32]. Infectious reovirus subviral-particles, induce the formation of multisized channels that are permeable to anions [32]. In fibroblasts that express the fusogenic hemagglutinin molecule from influenza virus, 1–2 nm wide pores are formed [30].

4. Translocation of proteins through membranes requires energy

The insertion of diphtheria toxin into a membrane is not, by itself, sufficient for the translocation of the toxin's fragment A to the cytoplasm since energy is required to push this protein moiety through the membrane. This energy is provided by a protonmotive force

[33], a mechanism used also for translocation of other proteins across membranes [34–36]. Still, other toxins, as the ricin A chain, are translocated by a pH-independent mechanism [37]. Energy in the form of ATP may be, instead, required to translocate the ricin A chain [37].

In addition to the above energy requirements, protein translocation is associated with conformational changes in the protein, which allow its interaction with the membrane and its subsequent translocation to the cytosol. It seems possible that virus entry may be mechanistically similar and may be dependent on the existence of a pH gradient, membrane potential, or ATP to translocate the genome (or the nucleocapsid) to the cytoplasm.

5. A protonmotive force model for animal virus entry

A protonmotive force model (the chemiosmotic hypothesis) was presented a number of years ago by Mitchell [38,39] and states that special transducing enzymes can use a proton electrochemical gradient to synthesize ATP in cells. The translocation of protons through biological membranes to generate such a gradient, requires energy, but the dissipation of this gradient produces energy that can be coupled to non-favorable thermodynamic processes.

A pH gradient is generated in endosomes by the activ-

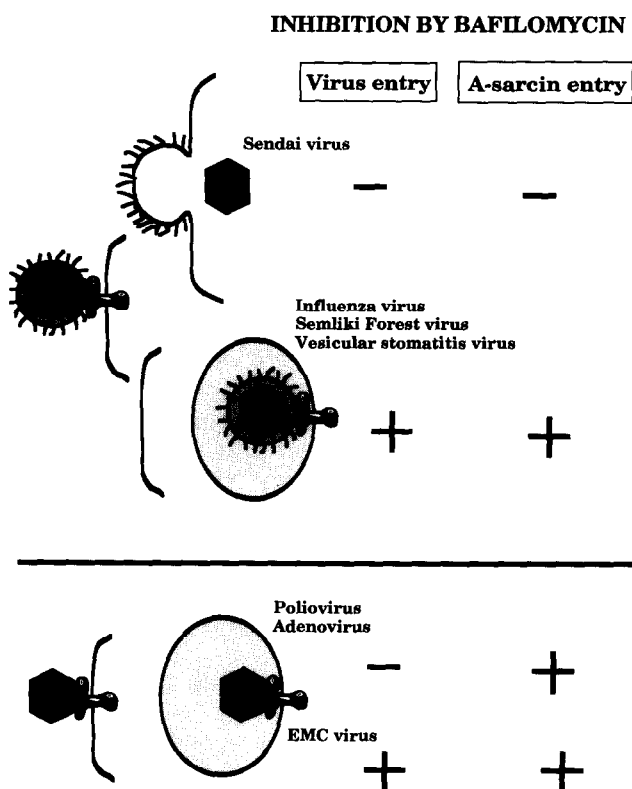


Fig. 2. The use of bafilomycin A1 to test virus entry and the co-entry of the toxin alpha-sarcin indicates the route of entry of a particular animal virus. The figure summarizes the results already known [17–19].

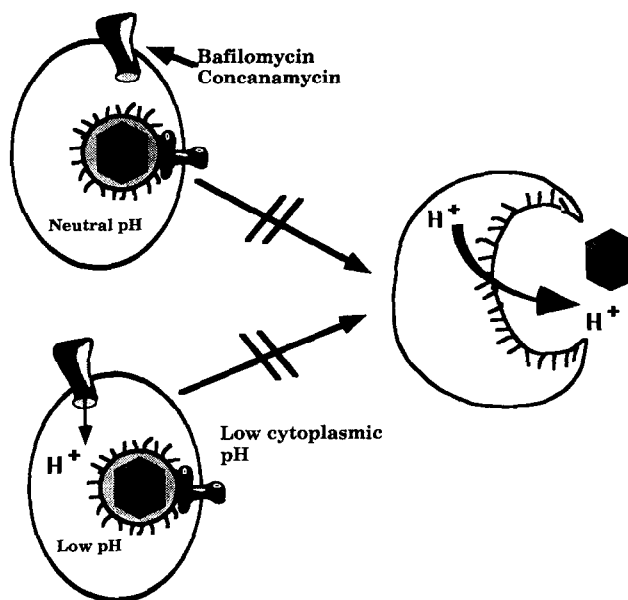


Fig. 3. Schematic representation of the protonmotive model for animal virus entry into cells. The release of the virus nucleocapsid to the cytoplasm by enveloped animal viruses that enter through endosomes, requires the dissipation of the proton gradient. Accordingly, entry would be blocked, not only by increasing the pH in endosomes by inhibitors of endosome function, but also by decreasing the pH in the cytoplasm.

ity of the vacuolar proton-ATPase pump. Recently, we found that this activity is required both to promote virus entry and to drive other macromolecules through the lipid barrier of the membrane [17,18]. Thus, I propose a new model for animal virus entry that takes into account the experimental evidence now available. The model implies that the viral proteins inserted in the membrane may couple the energy liberated by the movement of protons (or ions) to the cytoplasm, in favor of its gradient, to the translocation of the viral genome (or protein toxins) in the same direction. Therefore, the energy accumulated in endosomes in the form of a pH gradient and/or membrane potential is required for the viral nucleocapsid to enter cells. In addition to virus entry, the early permeabilization phenomenon observed with virus particles can also be easily rationalized by this protonmotive force model. Thus, viral proteins involved in the translocation of the viral particle across the membrane would also translocate other macromolecules, using an existing pH gradient or membrane potential. Therefore, the toxin moieties involved in the translocation of toxins through membranes behave like virus particles.

This model implies that certain viral proteins open pores in membranes and harness energy for the translocation of substrates in a non-favorable thermodynamic direction. Whereas uncoupling agents dissipate the energy stored in ionic gradients, the 'virus transducing complex' either couples this energy to genome transloca-

tion or uses it to promote the passage of proteins or protein–nucleic acid complexes to the cytoplasm.

Additional support for this model is provided by the fact that virions modify membrane potential during entry (see reviews [12,27], probably as a consequence of the capacity of virion proteins to form ion-channels [30–32]. The entry of SFV does not occur when the membrane potential is abolished by modifying the concentration of monovalent cations, even under acidic conditions [40]. Even though viruses can attach to dead cells (and even to isolated membranes, or truncated receptors) [8,41], they only effectively fuse with and enter into cells that possess an energized membrane [27].

This model would particularly apply to those animal viruses that require a low-pH step to infect cells. In this instance the virus needs the pH gradient to enter. Viruses that enter cells by a pH-independent mechanism could rely on the existence of a membrane potential, or be able to directly use ATP as the energy source to translocate their genome into the cytoplasm. Therefore, it is possible that different viruses use different mechanisms for genome translocation, the point is to determine if all of them require energy during this step of virus infection.

The mechanical and the protonmotive force models can be differentiated experimentally. The low-pH model, that explains the entry of enveloped viruses into cells, indicates that a low pH is sufficient to allow virus fusion and entry in a mechanistic way. Thus, low pH changes the conformation of a protein that is inserted into the cellular membrane and fusion ensues. The protonmotive force model indicates that low pH is not, by itself, sufficient for virus entry. Thus, under low pH conditions virus infection will be blocked if the pH gradient is destroyed (see Fig. 3). Alterations in the cytoplasmic pH or ion concentration also interfere with the endocytotic process [4,5,42]. Therefore, destruction of the pH gradient may be required for virus genome translocation and for an intact endocytic pathway. Future studies on this exciting field of virus entry, which will be directed towards elucidating the exact molecular basis underlying both virus genome delivery into cells and the early membrane permeabilization that is induced by virion particles should indicate which of these models is the closest to reality.

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