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Research Proposal: Structure of the RNA Channel Formed between Poliovirus and the Afflicted Cell During Infection

Introduction

Recent work by Bubeck et. al. (1) has furthered elucidation of the pathway by which poliovirus enters a cell. In particular, this work calls into question the conventional model by which the entry of the poliovirus RNA genome into the infected cell is understood. Detailed structural analyses prove that in the intermediate 135S stage, the VP1 and VP4 protein subunits of the poliovirus somehow interact with the plasma membrane of the cell to be infected, thus forming a channel through which the viral RNA can travel (1). This step, which must be initiated by the binding of the nucleocapsid to the poliovirus receptor (Pvr) on the cell's surface, is crucial for the infectivity of the virus. However, once a virus has reached the 135S stage, it no longer requires binding to Pvr to cause infection (2).

A relevant question now is how exactly this nucleic acid channel is formed. Several models have been set forth, each proposing different roles for VP1 and VP4 in channel formation (1). One model hypothesizes that the amphiphatic helices of multiple VP1 subunits will form a 5 helix bundle which serves as the channel through which the viral RNA can pass. However, structural analysis shows that there is probably not enough room for such a large helical bundle to form, and additionally, mutational analysis has shown that VP4 plays at least a somewhat more active role in channel formation (1, 3). Accordingly, other models have been proposed. One is that VP4 is the central player in channel formation, and that VP1 serves to simply anchor the poliovirus nucleocapsid to the plasma membrane (1). And finally, a third proposed model is that both VP1 and VP4 play a role in the formation of the RNA channel (1).

Figure removed due to copyright reasons. Please see figure 6 from:
Bubeck, Doryen, et al. "The structure of the poliovirus 135S cell entry intermediate at 10-angstrom resolution reveals the location of an externalized polypeptide that binds to membranes." *J Virol* 79 (2005): 7745-7755.

<u>Specific Aims</u>

The aim of these experiments is to elucidate the means by which the poliovirus creates a channel for its RNA genome to pass from the nucleocapsid into the cytoplasm of the infected cell. More precisely, the experiment aims to show first of all whether the functions of VP1 and VP4 are exclusively to anchor the nucleocapsid to the cell, or exclusively to provide a channel for the passage of RNA through the plasma membrane, or whether it is a combination of both functions. A second aim of the experiment is to directly visualize the passage of the viral RNA through its channel, and by doing so to visually ascertain the composition of this channel.

All in all, these experiments will test the hypothesis that VP4 alone forms the nucleic acid channel, with VP1 anchoring the nucleocapsid to the plasma membrane.

<u>Experiments and Expected Results</u>

Mutational Analysis:
>   To test the possible role of VP1 in channel formation, all of the residues in the amphiphatic helix of VP1 will be changed into polar and/or charged residues. If VP1 does indeed normally form the channel for the RNA, then this would prevent the channel from crossing the membrane, and would prevent pathogenesis. If VP1 is an essential anchor holding the nucleocapsid to the plasma membrane by embedding itself in the nonpolar region of the membrane, then this mutation would also prevent pathogenesis. However, if instead VP1 anchors the nucleocapsid to the plasma membrane by electrostatic interaction with the polar phospholipid heads of the fatty acids in the lipid bilayer, then this mutation would not prevent pathogenesis.

>   To distinguish between these three possibilities, a second test is required. This time, all the residues in the amphiphatic helix of VP1 should be changed into nonpolar residues. If VP1 does form the channel for the RNA, then this mutation would prevent pathogenesis because the polar RNA could no longer pass through this channel. If, on the other hand, VP1 is an anchor, then infection should continue if the poliovirus is anchored by embedding itself in the plasma membrane, whereas infection will be prevented if the anchor is via electrostatic interaction with the phospholipids.

>   This leaves 4 possibilities:
>   - Experiments 1 and 2 create pathogenic particles: VP1 may be non-essential for channel formation.
>   - Only Experiment 1 creates pathogenic particles: VP1 may be anchored to the polar phospholipid heads of the fatty acids on the exterior of the lipid bilayer.
>   - Only Experiment 2 creates pathogenic particles: VP1 may be anchored within the non-polar interior of the lipid bilayer.

- Neither Experiments create pathogenic particles: VP1 may serve as a channel for the RNA to pass from the nucleocapsid of the virus into the cytoplasm of the cell being infected.

The same experiments are then repeated with VP4 to independently assess its role in RNA transfer.

To do these experiments, wildtype 135S poliovirus is propagated and prepared as previously described (4). Mutagenized versions of the sequenced 135S poliovirus are prepared by PCR amplification of the poliovirus genome using mutated primers (site-directed mutagenesis), and then by repeating the process of virus propagation and preparation. After harvesting native and mutagenized 135S poliovirus, their structures are first examined with cryo-electromicroscopy (as described previously) to ascertain that the introduced mutations do not prevent proper virus assembly. The pathogenicity of these virus particles is then tested by attempting to infect HeLa cells. Failure of a mutagenized poliovirus to infect a population of cells when wildtype is capable of doing so indicates that the mutagenized portion of the genome was essential for not only pathogenesis, but also for transfer of RNA, because this is the only remaining step for a 135S particle to infect a cell.

Visualization of RNA transfer:
Prepare wildtype 135S poliovirus as previously described (1). Then prepare very small liposomes (as previously described) such that they are too small to contain an entire poliovirus genome (5). Because the 135S only needs a plasma membrane to initiate pore formation and RNA transfer, without even Pvr, the wildtype 135S poliovirus particles should form RNA channels with the liposomes. However, because the liposomes are too small to contain the entire poliovirus genome, the transfer of the RNA will stall midway through. These stalled complexes can then be visualized by either cryo-electromicroscopy, or by staining the RNA with RNASelect green fluorescent cell stain and by staining VP1 and VP4 (or potentially other proteins as well) with different colors via immunofluorescence. A negative result would show viral particles fully of their RNA, and liposomes devoid of RNA without any viral particles attached.

This should allow the straightforward visualization of poliovirus while its RNA channel is still intact. Presumably this will provide a more direct test for the role of VP1 and VP4 in pore formation and will complement the data obtained through mutational analysis.

Discussions and Conclusions

The purpose of these experiments is to clarify the exact mechanism by which poliovirus forms channels with cells it is about to infect so that it can transfer its RNA. This is a poorly understood mechanism, although it is used not only in the picornavirus family, but also in the rather large family of viruses which do not have an enveloped nucleocapsid.

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By understanding this pathway, future work can investigate the potential of using pharmaceuticals to target the entry pathway to develop treatments for infected patients.

References

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