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Towards new structural insights into the process of Poliovirus RNA translocation upon infection

Introduction

Poliovirus is not only a significant human pathogen but also serves as a well-characterized model system for understanding how nonenveloped viruses infect cells. Upon binding to its receptor at the membrane of human macrophages, Poliovirus undergoes an irreversible conformational change that leads to penetration of the host cell membrane. Thereafter, it translocates its single stranded RNA genome through a self-induced pore into its host cytoplasm. The emptied nucleocapsid remains outside of the infected cell, which is then taken over by the viral replication machinery.

The icosahedral Poliovirus nucleocapsid is composed of 60 copies each of four coat proteins, VP1, VP2, VP3 and VP4. The virion surface is dominated by two structural features: star-shaped mesas at the five-fold axes and three-bladed propellers at the threefold axes. Upon binding to its receptor, the so-called 135S cell entry intermediate is formed, which involves a major conformational rearrangement that results in the externalization of VP4 and the N-terminus of VP1. Both, VP4 and VP1 are discussed to be involved in channel formation allowing the translocation of the viral RNA (TOSTESON AND CHOW, 1997). The N-terminus of VP1 resembles an amphipatic α -helix that is known to tether the particles to membranes. Several copies of this helix are believed to form the pore that allows the passage of the RNA. The contribution of VP4 remains poorly understood.

Very recently the structure of the 135S particle was reported by reconstructing it from cryo-electron microscopy data (BUBECK *et al.*, 2005). The externalization site for the N-terminus of VP1 was located near the propellers at the threefold axes. This was a surprising finding since it was believed that VP1 would be externalized at the fivefold axis, so that five VP1 helices could form the pore that clears the way for the RNA which is believed to leave the virus particle through an opening at the fivefold axis. Bubeck *et al.* suggest a new model in which VP4 constitutes the RNA-conducting channel whereas the N-terminus of VP1 acts either as a membrane anchor or acts in concert with VP4. Clearly there is a demand for a better understanding of the exact process of RNA translocation during Poliovirus infection.

Specific Aims

We will address in this proposed study the ill-understood process of RNA translocation in Poliovirus infection in finer detail. Anticipated is a cryo-electron microscopy structure of a Poliovirus virion during RNA translocation. Therefore infection has to be stalled at a precise time point namely when the RNA protrudes from the virus capsid through the viral channel into the cytoplasm of the host cell. We will achieve this by crosslinking the RNA to the viral protein during the translocation process. Since the RNA is covalently linked to the viral translocation machinery after crosslinking, the process of infection is halted at this time point and the particles can be studied by means of cryo-electron microscopy. In parallel, we will engage biochemical methods in order to support and widen our findings.

In particular, we will address two open questions with this approach. Firstly, the structure of Poliovirus during RNA translocation will reveal the opening in the nucleocapsid through which the RNA leaves the virus. Secondly, the nature of the RNA conducting channel will be understood more significantly. It will become clear if VP4 or VP1 forms this channel since it will be linked to the RNA as well. It might even be possible to preserve the structure of this channel for cryo-EM studies. Generally, a better understanding of the precise mechanism of Poliovirus infection can be anticipated.

Experimental Procedure

RNA can easily be crosslinked to proteins in its near proximity by irradiating it with a short laser pulse of ultraviolet light (for a review see PASHAW *et al.*, 1991). The overall structure of the crosslinked RNA-protein complex does not substantially differ from the native structure and can therefore be engaged for structural studies.

In this project, it is intended to infect human HeLa cells with native Poliovirus and to stall the process of infection by irradiating the virions during infection with UV light, therefore crosslinking the translocating RNA to both the nucleocapsid proteins and the viral RNA conducting channel.

First it has to be optimized in which conditions and after what time period of infection the crosslink should occur in order to yield a maximum amount of stalled RNA translocating virions. Of particular interest is here the ratio of viral particles to HeLa cells. We want to maximize the amount of translocating virions, whereas we want to minimize the amount of free, unbound virions. Next, it will most likely be useful to cool the infected culture down to 0 °C in order to slow down RNA-translocation and to maximize the target for crosslinking. Most probably a centrifugation step will have to follow at this timepoint, since the crosslink can only be accomplished in a small volume, so that the irradiation can hit the RNA molecules effectively. After the crosslink there shall be three different kind of viral subpopulations: 1.) Virions that did not start

infection, 2.) Virions that are caught during RNA translocation and 3.) Empty nucleocapsids that have succeeded in RNA translocation. Upon dissolving the host cell membrane with detergent the second subpopulation is distinguishable from the two others since it is the only subpopulation that has a protruding RNA attached to it. This difference can not only be employed for recognizing it by hybridization to a labeled RNA probe, but also to separate it from the other two since it will bind more tightly to anion-exchange materials.

After optimizing the crosslinking conditions, it has to be tested how to purify the RNA translocating virions in an especially mild manner. It is desired to keep the RNA conducting channel in its native state during purification so that it can be investigated by cryo-electron microscopy. This could be achieved by purifying the virions away from the host cell membrane in the presence of mild detergents. The detergent would preserve the hydrophobic interactions between the channel and the membrane therefore holding the transmembrane components in its native conformation. The purified particles will then be amenable for cryo-EM studies. While it is likely that this study will produce a detailed insight into the conformation of the nucleocapsid during RNA translocation, it cannot be predicted if the structure of the RNA conducting channel can be resolved out of the resulting images. In any way, every obtained secondary structure element of the RNA-conducting channel proteins will be helpful to distinguish between VP1 and VP4, because the first is composed of α -helices and the latter of β -sheets.

However, the structure of the components of this channel might be too heterogeneous for cryo-EM analysis. By employing biochemical methods it will at least be possible to identify whether VP1 or VP4 forms the channel, either of which will be covalently linked to the RNA. By digesting the RNA with nuclease the nucleocapsid/RNA particle can be separated from the channel/RNA particle. The protein component of the channel can then be identified by mass spectrometry sequencing. Certainly, the experiment will have to be scaled up for these kinds of experiments, since microgram amounts of proteins will be needed. While 100 mL of infected HeLa cell culture will be sufficient for cryo-EM studies, the required amount for biochemical studies lies in the range of 10 L infected HeLa cell medium.

Further cryo-EM studies will be performed on the emptied virion particles that have already released their RNA. We are convinced, that they will still be in the conformation in which RNA translocation took place. They can readily be purified and a cryo-EM structure in high resolution can be presumed. In comparing this structure to the previously reported 135 S structure much can be learned about the last conformational remodeling switch the virus undergoes before translocating its RNA.

Summary

By crosslinking the RNA of Poliovirus to its proteins, the process of RNA translocation can be stalled. The structure of the RNA translocating virion becomes therewith amenable for high-resolution cryo-EM studies. Even so the RNA translocating channel might not be able to preserve in its original conformation, biochemical approaches will be able to identify the proteins that line the RNA channel. Furthermore, a cryo-EM of the empty nucleocapsid after RNA translocation will clearly be amenable.

Out of this proposed projects much can be learned regarding the exact mechanism of how Poliovirus is enabled to enter its genome into a human cell. It will not only be observed where the viral RNA exits the nucleocapsid shell but also which protein constitutes the channel that permeates the host cell membrane.

References

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