

## Lipid Raft Incorporation in the Measles Virus Budding Mechanism

by

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### Abstract:

Lipid rafts incorporated in membranes are glycosphingolipid rich microdomains that have been found to be involved in infectious diseases. The paper discussed here investigated the role of lipid rafts in the budding mechanism of the measles retrovirus. Specifically, it focused on the individual proteins in the measles virus and their interactions with the lipid rafts individually as well as collectively. Results lead to a proposed mechanism of budding for the measles virus, the purpose and need for the lipid rafts, as well as an explanation of how the specific proteins interact with the lipid rafts and why.

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### The Measles Virus:

The measles virus, from the family *paramyxoviridae*, genus *Morbillivirus*, and species Measles Virus, is a viral illness that is now routinely immunized against in all school-aged children. Work has recently been done on the virus to determine how lipid rafts are involved in the viral budding mechanism. The virus as an infection is not a serious concern globally since the immunization was created; however, it does serve as an excellent model for studying lipid rafts and their role in different biomedical cellular and molecular mechanisms. Since so much about the virus

is already known, it is much easier to focus on the details of its lifecycle. (1,2,3)

Measles is an airborne virus that initially attacks the mucous membranes of the upper respiratory tract epithelium. Once in the respiratory tract, it will multiply and move to lymph nodes, eventually spreading to the blood. Its concentrations will peak in the infected body around 11 to 14 days after the initial contraction of the virus. Measles will spread through the air via coughs or sneezes as well as direct contact with infected nasal and throat secretions. It will present initially as a rash under the tongue, leading next to a generalized rash over the body, specifically in the head region. This can lead to itching

and flaky skin. Patients will have this rash along with a high fever, runny nose, red and watering eyes and a cough. If allowed to continue, it will become more serious, leading to the inability of the GI tract to absorb nutrients, resulting in malnutrition, diarrhea, and dehydration. Viral pneumonia, specifically ear problems, and brain damage can also occur, eventually ending in death if the disease is not properly treated. A central mechanism in the viral pathway is the inhibition of the host's immune response, allowing the virus to proceed without being attacked by the body. Measles will inhibit monocytes from releasing Interleukin-12, which normally stimulates cell-mediated immunity, a very important defense mechanism against viruses, bacteria and protozoa. Without this method of defense, the virus is able to proceed with multiple divisions and increase its level of attack. (1,2,3)

The measles virus is a single strand of RNA enclosed in a viral envelope. The envelope consists of a lipid membrane with three associated proteins. The Matrix (M) protein lines the inner surface of the envelope, creating a protein coat on the inside wall. The Fusion (F), and Hemagglutinin (H) proteins are transmembrane proteins that are involved in the fusion of the virus with the host cells and the absorption of the viral RNA into the host cell. These three cells will interact at the lipid raft to facilitate budding. The RNA is surrounded by a nucleocapsid that is made up of three more proteins. The Nucleoprotein (N) covers the RNA entirely, providing protection. The Large protein (L) is an RNA polymerase that will replicate the genome. The phosphoprotein (P) is a cofactor that helps the L protein in the replication of the genome. (1, 2, 3)

The viral mechanism involves the binding of the virus, the subsequent replication and eventual budding. Measles will bind to the

CD46 cellular receptor and undergo endocytosis. The endosome that is produced inside of the cell will then be uncoated and the viral RNA will enter the nucleus. Replication and transcription will occur, mRNA for the six viral proteins will be produced, and the cellular ribosomes will read the mRNA, translating the R, L, N, M, H, and F proteins. The N, P, and L proteins are transported to the nucleus where they are complexed with the viral RNA. This nucleocapsid will then leave the nucleus, complex with the M, F, and H proteins and form the viral particle. Finally, it will undergo the budding process to leave the cell. (1, 2, 3, 4)

#### **Measles Virus and Lipid Rafts:**

Some of the research being done on the measles virus is focused on this budding mechanism and how it incorporates lipid rafts. A recent study from the Journal of Virology, November 2000, did some of the pioneering work on lipid rafts in Measles viral mechanism. This study, "Measles Virus Assembly within Membrane Rafts", done by S. Vincent, D. Gerlier, and SN. Manie, focuses on determining which of the 6 proteins in the measles virus will intrinsically migrate to specific lipid rafts, thereby facilitating budding. (4)

A lipid raft is a glycosphingolipid-rich microdomain, which has self-association and cholesterol association characteristics. These characteristics will create a liquid ordered membrane domain. These domains are easily isolated as they are insoluble in ionic detergents at 4°C. They will float in a sucrose gradient and in this way, can be extracted from a sucrose gradient. Cells that have been infected by the measles virus have been dissolved in non-ionic detergents and the lipid raft domains that are extracted show the involvement of measles virus proteins with the lipid rafts. Conversely, measles virus particles isolated show lipid

raft characteristics in their viral envelope. It is accepted, then, that the lipid rafts in infected host cells are involved in the budding mechanism. Defining which proteins come into direct contact with the rafts and how they work is a necessary step in determining how the lipid rafts facilitate budding. This study focused on defining these proteins. (4)

The materials and methods involved in this study were well planned and simple. HeLa cells were infected with the measles virus (MV) including all of its proteins, as well as an altered virus, a chimeric measles glycoprotein virus (MGV). This was a vesicular stomatitis virus G glycoprotein. This served as a measles virus that does not contain the F and G proteins. With this alteration, they were able to test whether the internal proteins would intrinsically localize on the lipid rafts as opposed to just following the external proteins. After an hour, the cells were washed and incubated with or without fusion inhibitory protein. At 24 hours, Measles virus cells were collected to obtain the virus protein expression. At 7 days, the MGV cells were collected for virus protein expression. Antibodies for all of the proteins except L (F, H, M, N, P) as well as antibodies for MV and MGV and CD55 and CD71 were used. The CD55 and CD71 were used as controls as well as for ways of determining where the lipid rafts are concentrated. CD55 is a protein that localizes on lipid rafts. Lipid rafts will be localized where this protein is found. This was a simple way of extracting the correct areas of lipid raft membranes from the sucrose gradient. The CD71 protein served as a control in determining H and F movement to rafts. (4)

The infected cells were lysed on ice with protease inhibitors and Triton X100. Sucrose and EDTA were added, then it was centrifuged for 16 hours. The column was fractionated from the top into 8 fractions and

the rafts were recovered from the 2, 3, and 4 fractions, according to the ability of the rafts to float in the gradient. An aliquot of each fraction was used for immunoblotting. The proteins were separated by gel electrophoresis and transferred onto a membrane. Immunoreactive bands were visualized by using secondary horseradish conjugated antibodies and chemiluminescence. (4)

The results showed that M and F will intrinsically localize at the lipid rafts, sometimes dragging the H protein as well. In the isolated fractions with all of the proteins present, the F, H, N and M proteins all were localized at the rafts in different, but all significant quantities. To determine whether the internal MV proteins (M,N) would localize in the absence of the external protein (F,H), the MGVM and MGVN antibodies were used together and in this case, similar positions were found at the lipid rafts. When expressed alone, however, only the M protein intrinsically moved to the lipid rafts. When F was expressed alone, it would also localize on the rafts, where the H protein would not. Therefore, it was decided that M and F proteins both have intrinsic motivation to move to and localize on the lipid rafts in the membranes. It was also decided that the F protein will drag the H protein to the rafts with it. However, the F protein will not drag M or N proteins to the rafts. A test was also done to determine whether or not the ribonucleoparticle (RNP), containing the complexed RNA with the N, P and L proteins would drag the H protein to the rafts. IN these cases, the H proteins still did not localize at the rafts, indicating that only the F protein can drag it to the membranes. (4)

F and M proteins, then were the only proteins that moved themselves to complex with a raft. Furthermore, the M and N proteins would only associate with the rafts when the viral genome was expressed. It

was proposed that the transcription of the genome and the subsequent formation of the ribonucleoparticle will stimulate the movement of the M protein to the lipid rafts. If this is true, the RNP is the trigger for moving the M protein to the lipid rafts. This protein, if the RNP is already formed, is already complexed to the RNP, thereby essentially bringing the virus to the membrane to be further complexed with the F and H proteins. The F protein will interact with the H proteins and M will interact with the cytoplasmic tail of F. F and H will be targeted to the basolateral side of the cell where the lipid rafts are aggregated. At this site, M will be targeted to the lipid raft, will associate with F and the virus will undergo a final aggregation. The data suggests that the lipid rafts are essentially platforms for the measles virus budding system. A pathway proposed is as follows: 1) the F protein will localize at the lipid rafts, 2) the H protein will be drawn to the lipid raft by the F protein 3) the RNP is complexed in the cytoplasm (N, P, L, M, RNA) 4) the formation of the RNP allows for M to be targeted to the lipid raft. 5) F and M will interact forming the final complex 6) budding will occur with the virus taking the F, H, and lipid rafts along with it as the viral envelope. (4)

This research was significant in determining which measles virus proteins will instigate the movement of the virus to the membrane for budding. Now that it is known which proteins are in control of the initial budding process, it is possible to proceed to determine what the actual mechanism is and why the lipid rafts are used as platforms. More specifically, what is it about the lipid rafts that make them an optimal platform for budding. Are they beneficial in avoiding the host immune system? Do they serve to make further virus replication faster or more efficient? These questions can now be explored through further investigation of this virus' particular process.

#### Resources:

1. <http://webs.wichita.edu/mschneegurt/biol103/lecture14/lecture14.html>
2. <http://www.stanford.edu/group/virus/retro/2000/measles.h>
3. <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/V/Viruses.html>
4. Vincent t al. "Measles Virus Assembly within Membrane Rafts." Journal of Virology, November 2000, pp9911-9915, Vol 74, No. 21.

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