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Understanding the role of Arenavirus envelope glycoprotein (GPC) in cellular entry and its inhibition

Sundaresh Shankar
University of Montana - Missoula, sundaresh.shankar@umontana.edu

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Arenaviruses belong to a family of enveloped viruses that cause acute to severe hemorrhagic fevers worldwide. They are classified as Old World (OW) or New World (NW) species based on their phylogenetic and geographic distribution. There are no effective treatments and licensed vaccines available for arenaviral infection. Hence, these viruses pose significant threat to public health and are considered as potential bio-terrorism agents. The sole treatment option for arenaviral infection is the use of a nucleoside analog, Ribavirin. Ribavirin treatment is only effective when given at early stage of infection and has shown significant toxicity to humans. Thus, there is a dire need for developing better therapies. The virus is transmitted to humans from its rodent reservoir by contact. The virus enters the host cell by binding to a cell-surface receptor. Upon endocytosis, the virion core is released into the cell by low pH-induced fusion of the viral and endosomal membranes, mediated by the viral envelope glycoprotein complex (GPC). This process is a potential target for therapeutic intervention. In contrast to other viral envelope glycoproteins, the mature arenavirus GPC is a tripartite complex comprising a stable signal peptide (SSP) in addition to the G1 and G2 subunits. Once the virus is endocytosed, upon lowering of pH in the late endosome, GPC undergoes a series of conformational changes that promote membrane fusion. We have found that the interaction between SSP and G2 plays a crucial role by mediating the pH at which fusion occurs. However, the detailed mechanism by which GPC is activated and facilitates/promotes pH dependent fusion still remains elusive. We believe that understanding this fusion event will provide support for novel drug development. Recently, chemically distinct class of small-molecule compounds specific to Old World and/or New World viruses have been discovered that inhibit arenavirus entry. In order to better understand the mechanism involved in viral membrane fusion and its inhibition, we carried out studies using recombinant Junín GPC (New World virus) produced in insect cells using a baculovirus expression system. We were able to successfully reconstitute GPC-mediated pH-dependent membrane fusion activity using a proteoliposome based fusion system. The fusion activity was specifically sensitive to inhibition by NW-specific inhibitors, e.g., ST-294 (SIGA Technologies). Genetic analysis carried out in our lab suggests that ST-294 acts by targeting the interaction between G2 and SSP. We confirmed that all the chemically distinct inhibitors share a common binding site by using a fluorescence-based competitive binding assay. To further map the site of inhibitor binding, our collaborators at The Scripps Research Institute developed photoactive inhibitors of the Lassa Fever virus (Old World) GPC. The recombinant Lassa GPC was photoaffinity labeled on insect cell membranes and the adduct was detected with a fluorescent signal. The results show that these compounds bind specifically to the mature G2 subunit or to SSP, but not to the GPC precursor containing the uncleaved G1G2 polyprotein. The photo-labelled amino acid residues will be determined using Tandem Mass Spectroscopy. By carrying out further studies using other derivatives with photo-groups at different positions, we should be able to map binding site, which will clarify the role of SSP and G2 in pH activation and mechanism involved in inhibition. This will guide us in lead optimization and ultimately opening up new opportunities in drug design and development.