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Review

Cell biology of virus entry: a review of selected topics from the 3rd International Frederick meeting

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Abstract

Following the first two Frederick meetings on virus entry in 1997 [Cell 91 (1997) 721][1] and in 2000 [Cell 101 (2000) 697][2], further developments in our understanding of the multifactorial and multistage process of virus entry, and possible biomedical implications were presented and discussed in a lively fashion by leading scientists from around the world at the third Frederick meeting on the Cell Biology of Viral Entry (May 7–10, Frederick, MD) organized by R. Blumenthal (NCI-Frederick, NIH, Frederick) and E. Hunter (University of Alabama, Birmingham). Unlike the previous two meetings, non-enveloped viruses were not discussed this time, and the focus was how envelope glycoproteins (Envs) mediate entry into cells. Major topics included Env structure, virus receptors, entry intermediates, membrane fusion, fusion kinetics, and rafts. Virus envelope structures will be described in more detail here because the other topics are extensively discussed in the other chapters of this volume.

1. Virus envelope structure and function

Binding to receptor(s) and changes in conformation, which are the ''heart'' of the entry machinery, are critically dependent on the virus 3D structure. Virus envelope structures were discussed at different levels: whole viruses, oligomeric Envs, Envs containing the receptor binding site(s), fusion proteins, and peptides mimicking fusion proteins. Stephen Fuller discussed extensively major features of the class II fusion proteins revealed by cryo-electron microscopy (cEM) that in combination with image reconstruction has provided three-dimensional structures of a whole virus (Semliki Forest Virus (SFV)) at 9 Å. cEM of SFV in isolation, and in the presence of liposomes of varying lipid compositions, provided wealth of information for the series of stages toward productive fusion and the rearrangements of the fusion protein during these stages. cEM faithfully preserves the structures of enveloped viruses and allows their visualization and interpretation of their three-dimensional structure. cEM also preserves their infectivity. This is important for the use of cEM with pathogens.

Mike Lawrence discussed the structure of the ectodomain of the fusion glycoprotein of Newcastle disease virus that has been determined to 3.3 Å resolution via X-ray crystallography. The observed molecule is a homotrimer with a club-like shape morphologically consistent with images via negative stain electron microscopy. He suggested that because the orientation of the central HR-A coiled coil could be in the opposite direction (with respect to the viral membrane) to that observed in influenza virus haemagglutinin (HA), the structural transitions accompanying fusion might be quite different for these two Envs.

Richard Wyatt and his collaborators have analyzed the crystal structure of the HXBc2 ''core'' gp120 exterior envelope glycoprotein in ternary complex with the primary viral receptor, CD4, and a neutralizing antibody, 17b. The structural analysis revealed a recessed CD4 binding site that spanned the two major domains of the gp120 glycoprotein. The CD4 binding site was characterized by several unusual cavities evident on the gp120 molecular surface. One of the gp120 cavities possessed critical contacts with the CD4

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residue Phe 43, which was positioned at the neck of the cavity. This cavity is highly conserved and has virtually an identical relationship to the Phe 43 CD4 residue in the recently solved YU2 core gp120 crystal structure. The primary isolate YU2 core glycoprotein has essentially the same fold as the HXBc2 core, with amino acid and glycosylation variability predominantly localized to the gp120 outer domain. Trimer modeling of the two structures indicates that this variable surface is the region of the envelope spike most exposed to the immune system and to potential neutralizing antibodies. Biophysical analysis by titration microcalorimetry indicated that CD4 binding to gp120 dramatically decreases gp120 entropy and likely locks gp120 into a distinct conformation. The flexibility may be a prerequisite for gp120 to perform its functional role in receptor binding and viral entry. Several structure-based mutants have been designed to lock gp120 into a state that mimics the low entropy, CD4-bound conformation. One such gp120 mutant, S375W, increases CD4 binding yet eliminates recognition by several CD4 binding site antibodies. The identification of conformationally fixed glycoproteins may provide unique molecules useful for the elucidation of gp120 structure/function, for the design of gp120-based vaccines and may provide insights for gp120-directed drug development.

Dennis Burton presented an analysis of the recognition of viral envelope glycoproteins by human monoclonal antibodies with an emphasis on how the recently solved crystal structure of IgG1 b12 helps our understanding of HIV neutralization. It appears that a major determinant of the potent and broad neutralizing activity of b12 is the long protruding finger-like CDR3 of its heavy chain that allows high affinity binding to native trimeric HIV envelope glycoproteins. He pointed out that mouse monoclonal antibodies might not have such long H3 as human monoclonal antibodies that could explain the fact that all potent broadly HIV neutralizing monoclonal antibodies are human.

Dimiter Dimitrov, who chaired the session on Viral Envelope Structure and Function, gave an overview of the history and the current rapid development of this area of research. For a very long time, the only structure that has been solved was that of influenza HA in Don Wiley's laboratory, and this was the complete trimeric ectodomain including both the surface (HA1) and transmembrane (HA2) proteins. The solution of the tick-borne encephalitis virus (TBEV) E glycoprotein crystal structure in 1995 initiated a new period of explosive development in the structural biology of envelope virus entry. For the last 6–7 years, a number of structures have been solved including portions of Envs responsible for binding (Friend MuV Env, HIV gp120), fusion (low pH influenza HA2, MoMuLV TM, HIV gp41, Ebola GP2, SV5F F, HRSV F, Newcastle Disease Virus F) and even whole viruses (SFV, TBEV particles, Dengue virus). Since 1995, the structure of the TBEV E glycoprotein stood alone being very different compared to that of HA. While numerous studies confirmed the original model proposed in 1993 by Peter Kim for the role of coiled coils in fusion for a number of viruses, until very recently, there were no other Env structures similar to that of the TBEV E glycoprotein. A major theme of discussion at the meeting was the recent proposition by Felix Ray for two classes of fusion proteins—class I based on HA, and class II based on the TBEV E glycoprotein structure. This proposition was based on the newly solved structure of the SFV E1 glycoprotein and more recently the one of dengue virus that exhibited structures and conformational changes very similar to those for the E glycoprotein without involvement of coiled coil structures that are critical components of the fusion mechanism mediated by class I proteins. Dimiter Dimitrov also briefly discussed recent data from his group on identification and characterization of broadly cross-reactive HIV neutralizing human monoclonal antibodies, and the development of tethered Envs where gp120 and gp41 are joined by flexible linkers of variable lengths allowing restricted exposure of entry intermediates.

2. Virus envelope glycoproteins and their receptors

Viruses have evolved to utilize cell surface molecules for gaining access into the cell interior. Receptor-mediated viral entry has been documented for Paramyxoviruses such as measles virus, respiratory syncytial virus, parainfluenza viruses, and retroviruses such as HIV, RD114 group of gamma retroviruses, and others. Edward Berger gave an overview of the various receptors involved in entry of viruses to cells including CCR5/CXCR4 for HIV and CD46 for Herpesvirus. James Hoxie described Env-coreceptor interactions in CD4-independent isolates of HIV-1 and HIV-2 that are able to directly interact with chemokine receptors as a result of mutations in the Envs including a highly cytopathic CD4-independent HIV-2 isolate and replication competent variants of HIV-2 lacking V1/V2 as well as major region of V3; these novel variants would also be tested for generating new immune responses to cryptic epitopes. John Moore discussed disulfide-stabilized HIV-1 Envs mimicking native cleaved Envs and their potential as vaccine immunogens. David Kabat described the RD114 group of gamma retroviruses utilizing Na+-dependent transporters as receptors and how sequence alteration in their extracellular loop 2 and variation in N-linked glycosylation of this region affects the host-range of this viral family. James Cunningham discussed cooperative mechanisms of infection by some retroviruses. Dusty Miller reported identification of a hyaluronidase family member Hyal2 as the cell-surface receptor for the Bovine retroviruses jaagsiekte sheep retrovirus (JSRV) and enzootic nasal tumor virus (ENTV), and its potential role as a tumor suppressor gene. Mark Goldsmith discussed coreceptor specificity and HIV pathogenesis.

3. Viral envelope proteins and fusion

This session was chaired by Paul Bates, and lectures were presented by Robert Lamb (Membrane fusion machines of paramyxoviruses: capture of intermediates of fusion), Min Lu (Attempts to define conformational transition in the HIV-1 Env), Yechiel Shai (The HIV-1 gp41 N-terminal heptad repeats play an essential role in membrane fusion), Christopher Broder (Membrane fusion tropism and heterotypic functional activities of the Hendra and Nipah virus Envs), Judith White (Activation of a model retroviral fusion glycoprotein), and John Young (Characterizing the receptor-priming and low pH-dependent mechanism of ASLV entry into cells).

4. Membrane fusion

This session was chaired by Fred Cohen and talks were given by Barry Lentz (Spontaneous lipid rearrangements leading to fusion: lessons from model systems), Michael Kozlov (Interplay of lipids and proteins in membrane fusion: theoretical analysis), Joe Bentz (Architecture of the influenza HA fusion pore), Andreas Hermann (Stability of the ectodomain of influenza virus hemagglutinin—a theoretical and experimental study), and Richard Epand (The thermal denaturation of influenza virus and its relationship to membrane fusion).

5. HIV kinetics and intermediates

This session was chaired by Leonid Chernomordik and talks presented by Robert Blumenthal (The HIV Env-mediated fusion reaction), Eric Hunter (Sensitivity of HIV-1 to fusion inhibitors is modulated by coreceptor specificity and regions of Env outside of HR1), Jacqueline Reeves (Sensitivity of HIV-1 to entry inhibitors correlates with Env:coreceptor affinity and coreceptor density), Gregory Melikyan (Evidence that folding of HIV-1 gp41 into a six-helix bundle coincides with or immediately follows the fusion pore opening), Hana Golding (Dissection of HIV-1 entry with neutralizing antibodies to gp41 fusion intermediates), and Anthony DeVico (Antigenic properties of the HIV Env during cell–cell fusion).

6. Endocytosis, rafts, and viral entry

It has been proposed that sphingolipids and/or glycosphingolipids (GSL) plus cholesterol associate laterally to form membrane microdomains termed rafts. Such rafts have been suggested to associate with specific proteins while excluding others, and to be involved in numerous cellular functions including membrane trafficking, cell morphogenesis, and signaling. Recently, the role of rafts for entry has been the subject of extensive studies. The session on rafts and endocytosis was chaired by Mark Marsh and presentations were given by Gary Whittaker (Role of protein kinase C and clathrin in SFV and influenza virus entry), Warner Greene (HIV Nef enhances viral particle entry into the cytoplasm contributing to increased viral infectivity and HIV pathogenesis), Margaret Kielian (Lipid interactions with the alphavirus membrane fusion protein), Joshua Zimmerberg (Effect of microdomain lipids and the transmembrane domain of influenza hemagglutinin on clustering in the plane of the membrane and on membrane fusion), Santos Manes (HIV-1 entry into the cell: rafting on the cell surface), and James Hildreth (Role of rafts for HIV entry).

7. Selected topics of lively discussions

This meeting is well known with the lively discussions on interesting and in some cases somewhat controversial issues. This year was not an exception and the discussions were frequently very animated. The recent proposition by Felix Ray for existence of a class II fusion proteins (class I is based on the HA structure) has generated a lot of excitement in the field. This proposition was based on the recently solved structures of the SFV E1 glycoprotein and the dengue virus. Thus, the Envs of these viruses joined the TBEV E glycoprotein which stood alone since 1995 to form a group with fundamentally different mechanism of conformational changes leading to entry where coiled coil structures play no role in contrast to their prominent role for entry of influenza, HIV, and a number of other viruses. Whether there will be class III fusion proteins remains to be seen.

In an interesting further development of our understanding of HIV fusion kinetics, Robert Blumenthal demonstrated how the slow kinetics of HIV entry could be of critical importance for the efficacy of some of the new entry inhibitors. The fusion kinetics itself is dependent on coreceptor density and envelope coreceptor affinities (Jackie Reeves). It appears that HIV entry is much more stochastic than, for example, influenza entry, and the time from binding to the major receptor, CD4, to binding the coreceptor could be on the order of minutes that would allow sufficient time for inhibitors that bind conserved structures exposed after CD4 binding to inhibit entry. One example is the CD4i antibodies that act through a post-CD4 binding mechanism.

At the previous Frederick meeting, John Young proposed a new mechanism of viral membrane fusion for the avian sarcoma and leukosis virus (ASLV) based on priming upon interaction with its receptor, TVA, followed by activation at low pH. At that meeting, there were interesting discussions about these possibilities with Paul Bates and Judy White being the major opponents. At this meeting, John Young presented new data supporting his original finding. Judy White showed data suggesting that lipid mixing in the case

of ASLV could occur at neutral pH after initial priming by Tva receptor.

In another lively discussion, Gregory Melikyan and Hana Golding attempted to resolve the issue whether the formation of the six-helix bundles (6HB) occurs simultaneously or before the formation of the fusion pore. Gregory Melikyan identified two types of pores, ''labile pore'' and ''robust pore'', based on their sensitivity to inhibitory peptides and relative density of coreceptor. His data suggested that pore creation, maintenance, and perhaps its growth are all controlled by formation of the 6HB. Hana Golding presented data that neutralizing antibodies targeting fusion intermediates, including those targeting 6HB, potently arrested fusion suggesting the presence of 6HB, which form before the pore formation. Kozlov and Chernomordik suggested that there might be other proteins outside the fusion pore formation region that might also play a role in fusion reaction. Yechiel Shai added another role to the N-terminal heptad repeat besides its role in the formation of coiled coil structure. He showed that it plays an important role in merging of membranes and works synergistically with the fusion peptide (FP) to promote the lipid mixing.

The laterally associated sphingolipids and/or glycosphingolipids (GSL) plus cholesterol form rafts, which could play a role in viral entry and budding. Santos Manes presented an interesting approach to study the role of rafts in viral entry. He made mutants of CD4 that partition in non-rafts compartment and showed that the mutant although was efficient in binding to Env, it did not allow any fusion. Similar, mutations in influenza HA effected raft partitioning and fusion phenotype. Based on these observations Josh Zimmerberg proposed the concept of a ring raft that glues together the transmembrane domains of HA to make the fusion complex a functioning machine. However, Gregory Melikyan and Judy White did not observe a correlation between HA raft partitioning and HA fusion phenotype. Depletion of cholesterol affects rafts. James Hildreth showed data that depletion of cholesterol using methyl beta cyclodextrin (mBC) or 2 hydroxy propyl-beta-cycodextrin prevented HIV infection. He proposed to use mBC as a chemical condom and the initial trials are ongoing. Many other interesting topics have been also discussed.

8. Future perspectives

The future remains bright for studies of virus entry. The trend for combining structural, molecular biological, biochemical, and biophysical methods will undoubtedly provide a better understanding of the molecular mechanisms of virus entry. Late stages of virus entry including uncoating should be better characterized. New virus receptors will certainly be identified. Development of novel inhibitors of virus entry is urgently needed. We will certainly witness a more complete understanding of cell biology of virus entry and, hopefully, the development of novel inhibitors, vaccines, and delivery tools.

Acknowledgments

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