2665-Pos Board B95

Development of Tools to Study Retroviral Gag Assembly on Giant Unilamellar Vesicles(GUV)

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The retroviral Gag polyprotein provides the principal driving force for virus assembly and budding from the cellular plasma membrane. The binding of Gag to the plasma membrane (PM) is governed by several mechanisms, including electrostatics, hydrophobics, Gag multimierization, and recognition of specific lipid head groups. To better understand how Gag interacts with the PM, control proteins GFP-poly(K/R)n (n=4/8/12 residues), were purified and used to compare Gag membrane binding to an electrostatic membrane binding protein. Preliminary protein-liposome binding experiments suggest that the anionic lipids, PS and PI(4,5)P2, contribute to the recruitment of polycationic proteins as expected. Unlike previous results, which showed that HIV-1 Gag responded strongly to cholesterol, GFP-poly(K/R)n responded weakly to cholesterol. PI(4,5)P2 enhances more liposome binding for retroviral Gag than for the control protein GFP-poly(K/R)n. This study may shed light on how the retroviral Gag protein interacts electrostatically with membranes, and recognizes specific lipids such as PI(4,5)P2.

Membrane binding is not a prerequisite for Gag multimerization, however, it might enhance the formation of Gag-Gag interactions. It is known that assembly of Gag occurs on the PM, but it remains unclear when and where Gag multimerization takes place, and if membrane lipid composition influences Gag assembly. To visualize by fluorescence microscopy the interaction of Gag with membranes and to detect Gag-Gag interactions, Rous Sarcoma Virus Gag is labeled with different Alexa-flurophores using Sfp synthase. Giant unilamellar vesicles (GUVs), composed of lipids similar to cellular inner leaflet lipid composition, were employed to study Gag assembly by measuring fluorescence resonance energy transfer (FRET). This study may provide a starting point for understanding where Gag multimerization happens as well as how membrane composition affects Gag assembly.

2666-Pos Board B96

Paradigm Shift in the Mechanism of HIV-1 Core Biogenesis

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The proteolytic cleavage of the poly-protein HIV-1 Gag, which is assembled on the surface of plasma membranes of infected cells, drives the conversion of the virus from the initial immature, non-infectious form to the functionally distinct mature, infectious form. Gag cleavage results in a series of structural changes, ultimately leading to the formation of a mature core. Current models for assembly of the mature core suggest that the cleaved HIV capsid protein (CA) nucleates in a concentration-dependent manner, and polymerizes forming the conical core in a diffusion-controlled process. These models also postulate that the core begins to grow at its narrow end, and stops growing once it reaches the membrane at the opposite end. Thus, the size of the virus itself is expected to be the primary factor that determines core size. Our findings challenge this view.

Cryo-electron microscopic analyses show that along with infectious viruses, viral isolates also comprise large membranous structures that contain multiple, freely-floating mature cores. Numerous instances of membrane-attached assembly intermediates with partially formed ''core-rolls'' that are at different stages of conversion from a planar Gag lattice to the mature core are also observed. These results indicate that the mechanism of core formation involves a non-diffusional, cooperative transition triggered by cleavage of the immature Gag lattice, resulting in its rolling away from the plasma membrane to form sheets that wrap around the viral RNA. Unlike the present models, our mechanism predicts that the generation of infectious HIV-1 will be severely affected by incomplete cleavage CA from the Gag matrix (MA), and that even a small percentage of uncleaved MA-CA would prevent the core from rolling away. This prediction explains previous experimental results; only 4% of cleavage-resistant MA-CA is sufficient to cause a 50% reduction of infectivity.

2667-Pos Board B97

Molecular Dynamics and Assembly Switch of FtsZ Filaments

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The cytoskeletal protein FtsZ assembles in a head-to-tail manner, forming dynamic filaments that are essential for bacterial cell division. Our studies using unbiased molecular dynamics simulations from representative filament crystal structures disclose different filament curvatures supported by nucleotide-regulated hinge-bending motions between consecutive FtsZ subunits, in agreement with experimental observations, and unravel the natural mechanism of the FtsZ assembly switch. Whereas GTP-FtsZ filaments bend and twist in a preferred orientation, thereby occluding the nucleotide binding site, the differently curved GDP-FtsZ filaments identified exhibit a heterogeneous distribution of open and closed interfaces between monomers. The key stabilization component involved in closing the interfaces in GTP-FtsZ filaments is the coordinated Mg^{2+} atom, which the opening GDP filaments lack. We have monitored, for the first time, the relaxation of the assembly switch, from the initial open inter-domain cleft conformation in filaments to the closed-cleft inactive conformation in unassociated monomers. Integrating this assembly switch and the nucleotide-dependent interfacial filament stability, our work offers a detailed molecular interpretation of the assembly-disassembly FtsZ cycle and its inhibition by antibiotic PC190723.

2668-Pos Board B98

Structural and Biophysical Comparison of UPEC and ETEC Adhesion Fimbriae

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Adhesion fimbriae (pili) of uropathogenic and enterotoxigenic Escherichia coli (UPEC and ETEC, respectively) facilitate adherence of the bacteria to target cells. Fimbriae are absolutely necessary for colonization and biofilm formation in the initiation of disease. The types of fimbriae expressed on the bacterial surface vary with the preferred environmental niche of the bacterial strain. For example, UPEC that express P-pili are most frequently associated pyelonephritis, an infection in the upper urinary tract, whereas bacteria that express type 1 fimbriae commonly cause cystitis through infection of the lower urinary tract. In contrast, ETEC expressing CFA/I and CS2 pili are associated with diarrheal diseases, initiating disease in the small intestines.

Although expressed in different enviroments, these fimbriae share basic structural and biomechanical features. Structurally, they are all long (1-4 μm), thin (7-8 nm diameter) helix-like filaments that extend from the bacterial surface. Biomechanically, they share the ability to be extended into a thinner filament (2-3 nm diameter) by unwinding of the helical filament under a constant force. However, the force required to unwind is specific to each fimbrial type. In addition, the dependence of the force required to unwind a fimbria on the velocity of this unwinding, (that is, the kinetics of unwinding), is also type-specific and highly variable. These biomechanical parameters are dissimilar for UPEC and ETEC expressed fimbriae, separating them into two distinct groups. Using force spectroscopy data, helical reconstructions from electron microscopy data, and computational simulations, we show in this work how these pronounced biomechanical differences may be beneficial for bacterial survival in a given environment.