BIOCHEMISTRY SEMINAR

A Reconstituted System for Biophysical Dissection of the Nipah Virus Fusion Cascade

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Abstract: Nipah virus (NiV) is a lethal enveloped RNA virus of the Paramyxoviridae family. The NiV fusion machinery, consisting of attachment glycoprotein G and fusion glycoprotein F, infects permissive cells through a pH-independent multistep membrane fusion event. NiV-mediated fusion is initiated by recognition of host cell membrane associated ephrin B2 receptors (EB2R) by NiV G, leading to conformational rearrangements in NiV G and subsequent triggering of NiV F by NiV G, that culminates in virus-host fusion. However, several aspects of the intricate molecular-level orchestration underlying NiV fusion cascade remain poorly understood, partly due to technical challenges in tracking fusion events in a complex cellular milieu. Here, we report a reconstitution-based approach for mimicking the NiV-host cell membrane interface, which enables quantitative fluorescence spectroscopic analysis of NiV-mediated membrane fusion. We find that this modular platform employing virus-like particles (VLPs) with a surface presentation of NiV glycoproteins and plasma membrane vesicles derived from host cells expressing EB2Rs is capable of successfully recapitulating NiV-host membrane fusion. We utilize this validated platform to perform a biophysical dissection of the NiV fusion cascade using hypo- or hyper-fusogenic F and G mutants that affect virus-host fusion at different intermediate steps. Collective insights from these experiments show that distinct facets of the NiV fusion cascade are differentially regulated by the F and G viral glycoproteins. These novel observations provide a quantitative mechanistic view of the regulation of virus-host fusion by the NiV fusion machinery that complements information from conventional cellular assays, and underscore the power and scope of this approach in exploring aspects of NiV fusion cascade not accessible to cell-based systems and techniques. Taken together, our work provides precise measurements of NiV fusion kinetics in a reconstituted system and enhances our knowledge of the molecular-level orchestration underlying fusion events orchestrated by NiV in particular and paramyxoviruses in general. Such a combinatorial approach utilizing reconstitution techniques, biochemical toggle switches for virus fusogenicity, and fluorescence approaches could serve as a blueprint for comprehensive analysis of the fusion behavior of viruses with complex multicomponent fusion machinery.

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