Early events in RNA virus infection: Uncovering interactions between host-encoded pioneer RNA binding proteins and the pre-replicated viral RNA of Chikungunya virus

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The success of an RNA viral pathogen to infect a cell can be defined by its ability to co-opt host factors, compete for endogenous gene expression machinery, and ultimately copy and re-package its genome at the expense of cellular resources and processes. Indeed, a virus that is able to replicate its genome marks a milestone in its lifecycle, having bypassed innate immune pattern recognition pathways and is on its way to gathering sufficient viral and host proteins for packaging and assembly. Thus, a particularly vulnerable phase of infection comprises of the steps prior to viral replication. Yet our molecular understanding of host-pathogen interactions, particularly between the viral RNA genome and cellular proteins that would interact with it, are lacking in large part due to an inability to distinguish interactions with first-generation viral genomes versus newly synthesized transcripts or subgenomic fragments. To specifically characterize interactions that occur between pre-replicated viral RNA genomes and cellular proteins, we developed a novel approach termed VIR-CLASP (Virus Induced Ribonucleoside-analog enhanced Cross-Linking And Solid-phase Purification). VIR-CLASP combines photochemical biology and RNA isolation methods with mass spectrometry to broadly identify proteins that directly interact with viral RNA only during the earliest events of infection, between nucleic acid entry and replication. Using this approach, we investigated early host-pathogen interactions during infection of human cells with Chikungunya virus (CHIKV), a zoonotic positive-sense ssRNA pathogen that is re-emerging as a global threat. We report the identification of hundreds of direct interactions between host cellular proteins and pre-replicated CHIKV genome, consisting of canonical RNA-binding proteins (RBPs) as well as non-canonical pioneer RBPs. Of these, we characterize and functionally validate the biological impact of three classes of RBPs, and find that they play critical antagonistic or facultative roles during replication – particularly in the first replication step, the synthesis of the minus-sense template strand of CHIKV. As VIR-CLASP does not rely on sequence-specific isolation of viral nucleic acids, our approach is utilizable to potentially all RNA viruses of interest.