432f Burst Size Distributions from Measurements of Single Cells Infected with Vesicular Stomatitis Virus

Ying Zhu and John Yin

Many RNA viruses, such as HIV-1, influenza A virus, and Hepatitis C virus, exist as genetically diverse populations due to their error-prone replication and short generation times. Increasing experimental and clinical evidence indicates that the broad distribution of genotypes within natural virus populations enable them to develop resistance to drug therapies, evade vaccination strategies, and play a role in the emergence of new viral diseases. However, it is not known to what extent distributions in virus-host phenotype, such as virus fitness or viral pathogenicity, reflect viral genotypes, host-specific variations or other environmental factors. A major technical challenge is that viral phenotype distributions are labor intensive to measure. Here we begin to address this issue by measuring burst sizes (virus fitness or progeny production) from single infected cells. As a model system we study vesicular stomatitis virus (VSV), an RNA virus that has potential applications for the production of pseudotype vaccines and as an oncolytic agent for the treatment of tumors. VSV has also served as a model for studying the evolutionary dynamics of virus populations, and it is of economic importance because it causes foot-and-mouth disease like symptoms in livestock. For our studies we employ rVSV-GFP, a recombinant strain of VSV that expresses green fluorescent protein (GFP) during infection, and we use fluorescence-activated cell sorting (FACS) to isolate single infected baby hamster kidney (BHK-21) cells early in their infection cycle. Analysis by plaque assay of virus produced from single GFP-positive cells showed a distribution of burst sizes from 0 to more than 5000 active virus progeny per cell, with typically 20 percent of GFP-positive cells producing no detectable virus. For multiplicities of infection of 0.1, 1 and 5, average burst sizes were 1300, 1200, and 1500 respectively, though distributions of burst size were not significantly different based on a one-way analysis of variance ($p=0.1249$). Further passaging of virus progeny from high- and low-yield cells and measurement of their distributions should reveal to what extent high- and low-yield phenotypes are transferable. By such means, we are aiming to identify how genetic and environment factors can quantitatively affect distributions of virus fitness.