VACCINIA VIRUS DNA REPLICATION AND VIRION MORPHOGENESIS: COMPLEX INTERPLAY OF VIRUS AND HOST

PAULA TRAKTMAN

Department of Microbiology & Molecular Genetics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Poxviruses are complex DNA viruses that replicate solely in the cytoplasm of infected cells, and therefore display an unusual degree of physical and genetic autonomy form the host. Molluscum contagiosum, monkeypox virus and variola, the causative agent of smallpox, are poxviruses associated with human disease. Vaccinia virus is the prototypic poxvirus for experimental research, as well as serving as the vaccine administered for protection against variola infection. Our laboratory has been involved in characterizing the processes of genome replication and virion morphogenesis and in investigating the contribution of dynamic protein phosphorylation to these processes. The viral B1 kinase plays an essential role in genome replication, but its precise contribution has been difficult to assess since it does not appear to modify any of the other replicative proteins. We have recently determined that B1 can rapidly and efficiently phosphorylate the cellular BAF protein, thereby eliminating BAF's potent DNA-binding activity. Cytoplasmic BAF appears to play an important role as a cellular defense against poxvirus replication. In the absence of active B1, cytoplasmic BAF associates with viral replication factories in a manner that correlates with the cessation of DNA synthesis. When B1 is active, BAF remains diffuse and replication progresses. Expression of a non-phosphorylatable derivative of BAF from the viral genome is lethal; in contrast, the temperature-sensitive phenotype of a *ts*B1 mutant is largely eliminated when infections are performed in cells lacking a cytoplasmic pool of BAF. Thus, the primary role of the poxvirus B1 enzyme is to combat the ability of BAF to bind to the cytoplasmic viral genome and sequester it in a fashion that is incompatible with replication. We propose that BAF serves as a sensor of exogenous, cytoplasmic DNA, and as such is an effector of a novel innate immune defense. The viral F10 kinase, in contrast, stands at the top of the hierarchy of viral proteins involved in the complex process of virion morphogenesis. Morphogenesis involves the diversion and remodeling of intracellular membranes into which viral membrane proteins have been inserted, the enclosure of proteins that comprise the virion core, and encapsidation of the virion membrane. The final particle contains ~75 proteins. Several components of the virion membrane and core are substrates of the F10 kinase, and phosphorylation is essential for productive virion assembly. Moreover, cellular protein-directed kinases appear to phosphorylate several components of assembling virions. In addition, we have shown that a number of cellular signaling systems are modulated by infection, and pharmacological perturbation of these pathways arrests virus production. Genetic, cell biological and biochemical analyses of the interplay between viral and cellular signaling systems in the process of morphogenesis will be discussed.

Keywords: orthopoxvirus, Vacinia virus, DNA replication