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# Murine Gammaherpesvirus 68 as a Model System to Study Virus-host Interactions

Jung-Jin Park[1], Sungbum Kim[1], Jin-Hyun Ahn[2], Seungmin Hwang[3], Ren Sun[3], and Moon Jung Song[1*]

[1]*Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University,*
[2]*Department of Molecular Cell Biology, School of Medicine, Sungkyunkwan University,*
[3]*Department of Molecular and Medical Pharmacology, University of California, Los Angeles CA90095, USA*

The subjects of our research are a group of gammaherpesviruses which are associated with malignancies. Epstein-Barr virus (EBV) is associated with endemic Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma, and lymphoproliferative diseases in immune deficient patients (1); Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus-8 is associated with Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease (2). The cause of these malignancies stems from the capacity of gammaherpesviruses to establish latent infection and evade from host immune surveillance besides transforming activities of certain viral genes. Due to limited host ranges of human gammaherpesviruses, we take advantage of murine gammaherpesvirus 68 (MHV-68 or $\gamma$HV-68), whose genomic arrangement and biological properties are closely related to KSHV and EBV, as a small animal model system to study the interactions of tumor-associated herpesviruses with the host and the molecular mechanisms that lead to pathogenesis (3-7).

Thus far, using the whole viral genome in a bacterial artificial chromosome (BAC) system, we have generated a random transposon mutant library of MHV-68, which nearly covers the whole genome (8). The library of mutant viruses was generated so that each virus has a distinctive sequence tag. A pool of mutants can be used to infect mice.

Comparison of the sequence tags on the pool of the viruses replicated in cell culture versus in one particular tissue, such as the lung, spleen, or brain will allow us to determine the viral genes specifically required for successful viral infection in different tissue. Since the members of the gamma herpesvirus subfamily share the similar genomic organization, our screening results will reveal important viral genes with previously unknown function. This method will be applicable to many different questions regarding viral infection *in vivo* and open a new avenue to understanding viral pathogenesis.

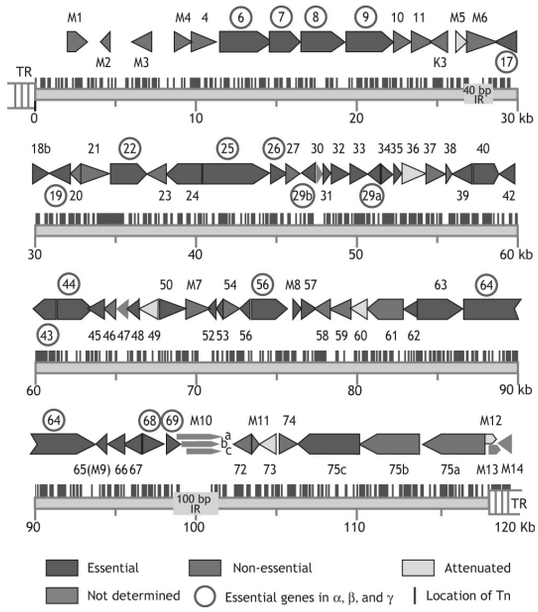


Fig. 1. Functional mapping of MHV-68 for *in vitro* growth. Putative ORFs of MHV-68 originally predicted by Virgin *et al.* (10) are color-coded, according to the growth properties of STM mutants with transposon insertion at the proximity to N-terminus of each ORF in fibroblast. The ORFs with rightward direction are named in the upper lines, while those with leftward orientation in the lower lines. The red circles indicate essential genes conserved in $\alpha$-, $\beta$-, and $\gamma$-herpesvirus subfamilies. The vertical lines represent transposon insertion sites, based on sequencing results of 988 mutants.

One of our aims is to dissect interactions between the virus and the host innate immune system. Interferons (IFNs) are a family of cytokines that exhibit such diverse biological effects as the inhibition of cell growth and protection against virus infection. Especially, type I IFNs (IFN-$\alpha$ and -$\beta$) are rapidly induced from virus-infected cells to initiate antiviral response, therefore serving as the first line of host innate immune defense against virus infection. Many type I IFN-activated genes bear a common responsive element in their promoters such as IFN-stimulated response element (ISRE). As anti-IFN strategies are present in many viruses, we set to test whether MHV-68 inhibits the activation of IFN-induced genes. Our results showed that wild-type virus infection reduced ISRE activation in NIH3T3 cells. Next, we employed genome-wide screens using our mutant library and a reporter cell line harboring a 5xISRE driven luciferase plasmid (5xISRE/3T3). Replication-competent STM mutant viruses were infected into 5xISRE/3T3 cells and screened for a mutant that restores IFN-induced ISRE activation to the level comparable to mock infection. Our results hint that MHV-68 has multiple immune evasion genes that may interplay in blocking IFN-induced ISRE activation at different steps of type I IFN signaling. Therefore, our systematic forward genetic approaches will offer unique opportunity to understand viral pathogenesis *in vivo* as well as *in vitro*.

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