Human cytomegalovirus (HCMV) is a herpesvirus that latently infects a majority of the global population. While infection is generally asymptomatic, HCMV can reactivate from latency and cause disease in immunocompromised individuals, including solid organ and stem cell transplant recipients. HCMV is also the leading cause of congenital infection and infectious disease-related birth defects in the United States. There is no vaccine, and current antivirals fall short in targeting the poorly understood latent infection. Understanding the interplay between viral determinants of latency and host factors is key to defining how latency is established and maintained in order to develop effective antiviral therapies. The goal of this work is to define virus-host interactions and determine molecular mechanisms of HCMV replication and latency.

Previous studies from the Goodrum Lab have characterized the UL133-UL138 gene locus of HCMV, which encodes four genes that modulate viral replication in order to establish, maintain, or reactivate from latency. UL138 suppresses viral replication and is required for latency in CD34+ hematopoietic progenitor cells (HPCs)--the primary reservoir for latency--but exact mechanisms are unknown. Preliminary studies identified interactions between UL138 and the host protein ubiquitin specific peptidase 1 (USP1) and its activator USP1-associated factor (UAF1). The process of ubiquitination involves a series of enzymatic reactions to add one (mono) or multiple (poly) ubiquitin proteins to a substrate protein in order to modify substrate activity or promote its degradation. While polyubiquitination often targets proteins for degradation, monoubiquitination can serve to activate proteins or regulate their trafficking, activity, or interactions. USPs reverse ubiquitin modification by cleaving ubiquitin moieties off substrate proteins. We have validated the UL138-USP1 interaction in infection. USP1 is a key regulator of the host DNA damage response (DDR) due to its role in deubiquitinating proliferating cell nuclear antigen (PCNA), a DNA clamp and processivity factor (promotes replication efficiency), among other proteins. <u>Thus, the UL138-USP1 interaction suggests a role for UL138 in modulating the host DDR to control virus replication and latency.</u>

In response to certain DNA damage stimuli, PCNA is monoubiquitinated (mUb). This modification terminates the interaction with bulk DNA synthesis polymerases (enzymes that add nucleotides to replicating DNA) and facilitates its interactions with translesion synthesis (TLS) polymerases. These are specialized low-fidelity DNA polymerases (e.g., pol iota, kappa, eta), that add nucleotides across the site of damage and allow for bypass in order to prevent the stalling or failure of DNA replication. Intriguingly, HCMV encodes its own DNA polymerase (UL54) and processivity factor (UL44), which is hypothesized to be similar in structure and function to PCNA. However, we have recently shown that host TLS polymerases are hijacked by HCMV and used to promote viral genomic integrity (Zeltzer et al, 2022). Much remains to be understood about how these proteins function during infection. Our preliminary data show that HCMV re-localizes PCNA to viral replication compartments in the nucleus, the sites of viral DNA replication. Further, mUb-PCNA protein levels increase throughout replicative HCMV infection and to a greater extent when USP1 is inhibited or depleted from cells. Using recombinant viruses, we show that UL138 has a specific role in stimulating deubiquitination of PCNA in infection, presumably through USP1. These findings suggest that the UL138-USP1 interaction modifies PCNA and, by extension, TLS polymerases during HCMV infection. Depletion of PCNA and TLS polymerases from infected cells indicate that these host factors restrict HCMV replication. Consistent with this, we have found that USP1 activity in hematopoietic cells is required for the establishment of latent HCMV infection. Given the roles of UL138 and its USP1 interaction partner as suppressors of virus replication for latency, <u>we hypothesize that UL138 regulates PCNA and TLS polymerases to restrict HCMV replication for latency.</u>

Aim 1 of this project is to mechanistically define how PCNA and TLS polymerases restrict HCMV replication. Using super-resolution microscopy imaging techniques, we will determine additional viral and host factors that PCNA and TLS polymerases associate with inside viral replication compartments. Genetic manipulation of defined interactions will reveal how these host proteins enhance or slow DNA synthesis rates.
Aim 2 is to determine the significance of UL138 modulation of PCNA and TLS polymerases to HCMV latency and reactivation. We will utilize recombinant viruses and commercially available drugs to assess how these host proteins impact viral latency and reactivation in HPCs. We hypothesize disruption of the UL138-USP1-PCNA-TLS pathway will prevent the establishment and/or maintenance of latency.

While many viruses induce DNA damage and hijack host repair proteins, the role of the DDR in HCMV infection has remained elusive. The identification of a viral protein, UL138, provides key insight and an innovative tool to mechanistically dissect the significance of DNA repair pathways to virus replication and how viruses have evolved to manipulate them for replicative and latent states.