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SUMMARY of
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Research Title		Structure-based mechanism of action of a viral PARP-1-interacting protein facilitating virus replication
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	Present Title	Structure-based mechanism of action of a viral PARP-1-interacting protein facilitating virus replication
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Summary

Poly (ADP-ribose) polymerase 1 (PARP-1), an enzyme that modifies nuclear proteins by PARylation, regulates various cellular activities and restricts lytic replication of oncogenic gammaherpesviruses by inhibiting the function of RTA, a key switch molecule of the viral life cycle. A viral PARP-1–interacting protein (vPIP) encoded by murine gammaherpesvirus 68 (MHV-68) orf49 facilitates lytic replication by disrupting interactions between PARP-1 and RTA. Here, we determined the structure of MHV-68 vPIP at 2.7 Å resolution. The structure consists of 11 α-helices with characteristic N-terminal β-strands (Nβ) and forms a V-shaped-twist dimer in an asymmetric unit. Structure-based mutagenesis revealed that Nβ and α1-helix (residues 2–26) are essential for the nuclear localization and function of vPIP; we then identified three residues (Phe5, Ser12, and Thr16) critical for vPIP function and interaction with PARP-1. A recombinant MHV-68 harboring mutations of these three residues showed severely attenuated viral replication both in vitro and in vivo. Moreover, ORF49 of Kaposi's sarcoma–associated herpesvirus also directly interacted with PARP-1, indicating a conserved mechanism of action of vPIPs. Our results elucidate the novel molecular mechanisms by which oncogenic gammaherpesviruses overcome repression by PARP-1, using vPIPs.

We determined the structure of full-length MHV-68 ORF49 (later called vPIP) at 2.7 Å resolution. The structure consists of 11 α-helices and characteristic N-terminal β-stands (Nβ) and forms a V-shaped-twist dimer in an AU. Three regions, L1 (amino acid residues [aa] 175 to 176), L2 (aa 231 to 237), and Ct1 (aa 280 to 300), were not visible in the electron density map of the crystal, suggesting that these regions have high flexibility. In our previous report, MHV-68 ORF49 was shown to interact with PARP-1 in a cellular environment. The results from surface plasmon resonance (SPR) analysis of the purified ORF49 protein regarding PARP-1–binding affinity in vitro indicated that KD is approximately 930 nM: sufficient affinity for PARP-1. This result clearly revealed the direct physical interaction of ORF49 and PARP-1; therefore, herein we propose to call MHV-68 ORF49 a viral PARP-1–interacting protein (vPIP).

The structure of vPIP was found to be a dimer in an AU, and its interface is formed by hydrogen bonds, salt bridges, and hydrophobic interactions. The salt bridges are R65A–E266B and K162A–D265B (each protomer is shown as a subscript). Size exclusion chromatography with multiangle light scattering (SEC-MALS) verified vPIP dimerization in vitro because the molecular weight (MW) was found to be approximately 76 kDa: a doubled MW of the vPIP monomer (38 kDa). Coimmunoprecipitation (co-IP) with an anti-FLAG antibody confirmed dimerization of vPIP in HEK293T cells cotransfected with FLAG-tagged vPIP and GFP-fused vPIP. These results indicated that vPIP exists as a dimer both in solution and in the cell.