THE PARVOVIRUS MINUTE VIRUS OF MICE MODULATES THE DNA DAMAGE RESPONSE TO FACILITATE VIRAL REPLICATION AND A PRE-MITOTIC CELL CYCLE BLOCK

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ABSTRACT

The DNA damage response (DDR) is a critical cellular network that affords cells the ability to repair DNA damage. Importantly, it has been shown that MVM *utilizes* this response to facilitate its replication in an ATM-dependent manner and induces a DDR-dependent pre-mitotic, G2/M cell cycle block *via* activation of Chk2 and depletion of the RNA and protein of the key mitotic cyclin, cyclin B1. Unexpectedly, this cell cycle block is p21-independent.

We investigated the loss of p21 during infection and found that siRNA knockdown of specific components of the $CRL4^{Cdt2}$ E3 ubiquitin ligase—Cul4A, DDB1 and Cdt2—both stabilized p21 and reduced viral replication during MVM infection. A stable p21 mutant that interacted with PCNA resulted in the significant depletion of MVM replication whereas a stable p21 mutant that no longer interacted with PCNA had no effect on replication. Taken together, this data suggests that MVM co-opts a cellular E3 ubiquitin ligase to target the CDK inhibitor p21 for degradation, which is required to allow the PCNA activity MVM needs for efficient replication.

To induce a pre-mitotic G2/M cell cycle block, MVM depletes the key mitotic cyclin, cyclin B1, which is preceded by the loss of its encoding RNA. Significantly reduced levels of RNA polymerase II (RNA pol II) and the key G2/M transcription factor, FoxM1, were found to occupy the cyclin B1 promoter during MVM infection. FoxM1, which requires hyperphosphorylation to activate its transcriptional activity, was found to have lower levels of phosphorylation during MVM infection. Reconstitution of FoxM1 to the cyclin B1 promoter upregulated cyclin B1 RNA and protein during MVM infection. Taken together, these results suggest that MVM prevents FoxM1 and RNA pol II binding to the cyclin B1 promoter, thereby reducing RNA pol II transcriptional activity and facilitating establishment of a pre-mitotic G2/M block.