

We have been studying parvovirus molecular genetics and parvovirus-host cell interactions productively for many years. Parvoviruses are important infectious agents, causing both persistent and acute infections of many animal species including humans. Additionally, parvoviruses, especially AAV, have been developed as effective gene therapy tools. Parvoviruses represent unique models to study essential aspects of DNA virus-cell interactions. Our work informs the parvovirus field about critical aspects of parvovirus infection, but also provides important insights into virus-host cell interactions and cell biology in general. We have been successful in making important advances in basic and applied virology and in areas of more general interest.

Although we continue to work on multiple aspects of parvovirus gene expression, much of our recent work has focused on how parvoviruses interact with the cellular DNA damage response. It has become increasingly clear that DNA viruses can provoke DNA damage responses (DDRs) in infected cells. These cellular responses are varied, but they are potent primary anti-viral responses, having the potential to impede or facilitate virus replication. Parvoviruses are the only known viruses of vertebrates that contain single-stranded linear DNA genomes, and they continually present novel replicative DNA structures to cells during infection. In contrast to the cellular response to genotoxic agents, in which cell cycle resumes after DNA repair, parvoviruses halt cell cycle progression and continue to replicate their genomes for extended periods of time in cells that remain arrested at the G2/M border. Over recent years we have been examining how MVM exploits the cellular DDR to prepare the nuclear environment for effective parvovirus takeover. We have shown that replication of the parvovirus minute virus of mice (MVM) induces sustained cellular DNA damage, and a vigorous DDR which the virus then exploits to enhance its infection in host cells. An essential aspect of the MVM-induced DDR is the establishment of a potent G2/M arrest. Although p53 remains activated, p21 is degraded and ATR/Chk1 signaling is disabled. We have recently shown that a surprising p21- and Chk1-independent cell cycle block is established *via* a novel mechanism that results in the significant, specific depletion of cyclin B1 and its encoding RNA, which precludes cyclin B1/CDK1 complex function preventing mitotic entry.

Additionally, there is currently much interest in how DNA viruses establish replication centers within the nucleus of cells. It is not clear how they situate themselves to optimize their replication – taking advantage of factors they require, and either evading or disabling inhibitory factors. There is an important connection inn this regard to the cellular DNA damage response. We have recently developed a generally adaptable, novel, high-throughput chromosome conformation capture assay for use in *trans* (we term V3C-seq) that allowed us to identify, on a non-biased, genome-wide basis, the specific, direct associations replicating virus genomes make with discreet regions of the cellular genome during infection. We term these sites Virus Associated Domains or VADs

This analysis showed the following. Initially, MVM VADs correlated with cellular sites that in mock-infected cells exhibited DNA damage as cells progressed through S-phase (likely early-replicating fragile sites). As infection progressed the VADs expanded, and as additional sites of DNA damage were induced, MVM subsequently also associated with the newly induced sites as infection amplified. Sites of association identified by V3C-seq were confirmed using super-resolution microscopy, and as an important test of our model, MVM could be targeted specifically to sites of DNA damage artificially engineered into the cellular chromosome (including both laser micro-irradiation “striping” and CRISPR/Cas9-induction of DNA breaks). Development of the V3C assay has allowed us to propose a new model for the initiation of infection of this small lytic virus. Soon after nuclear entry MVM homes first directly to sites of pre-existing endogenous DNA damage to initiate infection at these sites that maintain cellular

factors necessary for its replication. Subsequently, as cellular DNA damage accrues, virus spreads additionally to these sites of damage to amplify infection.