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Generation of a Mouse Model for Studying AAV Targeted Integration

Adeno-associated virus (AAV) replication has been extensively characterized both in vivo and in vitro. The nonstructural proteins of AAV-2 Rep78 and Rep68, in addition to the cis-acting 145 bp terminal repeat sequence, are essential for this function. Rep78 and Rep68 are multifunctional proteins with diverse biochemical activities, including site-specific binding to the AAV terminal repeat sequences, single-stranded site specific endonuclease, and helicase activity. In addition to lytic replication, AAV can establish a latent infection by targeted integration into chromosome 19 in the host genome. Located on ch-19 is a Rep binding element (RBE) and terminal resolution site (trs) identical to sequences identified in the viral terminal repeats required for DNA replication. Previous studies have determined that the ch-19 sequence requires both the RBE and trs for targeted integration in an EBV shuttle vector integration system. Currently, this sequence is present only in humans and monkeys. To better characterize the role of ch-19 cis-acting sequences in AAV targeted integration; a mouse model was generated by knock-in technology. Germline animals were generated and characterized for AAV targeted integration after infection both in vitro using embryonic fibroblast and in vivo after tail vein injection. Southern blot and PCR analysis confirmed AAV targeted integration in the ch-19 sequence located on the mouse X chromosome. In addition to providing an animal model for AAV targeted integration; we have initiated an effort to further define the critical Rep functions and TR cis-sequences involved in site-specific integration. A collection of AAV Rep mutants have been generated that lack specific biochemical functions (DNA binding, nicking, and helicase activity). Analysis of these mutants using a TR plasmid for integration and Rep supplied in trans, we determined that all three functions were essential. In addition, a TR mutant was constructed which is defective for in vitro nicking and in vivo viral replication. Analysis of this mutant for targeting is underway and the role of these sequences and Rep functions required for ch-19 integration will be discussed.

1982	Ph.D., Molecular Biology, University of Florida, Gainesville, FL
1982-1984	Postdoctoral Research Fellow, Microbiology Department, SUNY, Stony Brook, NY
1984-1986	Postdoctoral Research Fellow, Princeton University, NJ
1986-1992	Assistant Professor, Department of Biological Sciences, University of Pittsburgh, Pittsburgh PA
1992-1993	Associate Professor, Department of Biological Sciences, University of Pittsburgh, Pittsburgh PA
1993-1999	Associate Professor, Department of Pharmacology, University of North Carolina, Chapel Hill, NC
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Honors	
1990	Outstanding Young Men of America
1991	University of Pittsburgh, President's Distinguished Research Award
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