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RNF121 is a Key Transcriptional Regulator of AAV Genome Expression

Victoria J. Madigan[1,2,3], Julianne Yuziuk[2], L. Victor Tse[2], Anna Chiarella[1,5], Tyne Tyson[2], Nate Hathaway[5], and Aravind Asokan[2,3,4]

[1]Curriculum in Genetics and Molecular Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [2]Gene Therapy Center, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [3]Department of Genetics, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [4]Department of Biochemistry and Biophysics, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [5]Division of Chemical Biology and Medicinal Chemistry, Center for Integrative Chemical Biology and Drug Discovery, UNC Eshelman School of Pharmacy, Chapel Hill, NC, USA

A recent high-throughput screen for essential AAV host factors (Pillay et al., Nature, 2016) identified RNF121, a poorly characterized E3 ubiquitin ligase as a top hit in addition to AAVR. We investigated the role of RNF121 in the AAV life cycle by introducing mutations in the RNF121 gene using CRISPR/Cas9 in a panel of cell lines. Disruption of RNF121 reduced AAV transduction by over two orders of magnitude independent of cell type, AAV serotype or multiplicity of infection. Binding, cellular uptake, and subcellular trafficking assays indicate that AAV particles have no defect in trafficking to the nucleus in the context of the RNF121 knockout (RNF121KO) phenotype. Addition of proteasome inhibitors, transfection of Adenoviral (Ad) helper genes and co-infection with Ad failed to rescue the defective phenotype. Furthermore, transduction with recombinant Adenovirus was not altered by RNF121KO, suggesting this effect is specific to AAV. Transfection of the AAV genome with or without the AAV capsid added in *trans* was not affected, suggesting that association of the AAV genome with the capsid is integral to this phenotype. Transduction with self-complementary (sc) AAV vectors could not rescue the defective phenotype, suggesting second-strand synthesis is not impacted. Strikingly, RNF121KO causes a robust decrease in vector genome mRNA for both recombinant AAV vectors and wild-type AAV implying a defect in AAV genome transcription. These results were further corroborated through RNApol-ChIP. A number of host factors that might be regulated by RNF121 and directly affect AAV genome transcription have been identified by affinity purification mass spectrometry (AP-MS) to further investigate the mechanisms underlying AAV-RNF121 interactions. Our observations characterize a host factor critical for AAV genome transcription and corroborate the evolving notion that the AAV capsid plays a significant role in genome transcription.