Differential accessibility of chromatinised rAAV vector is a major regulator of variable expression in mouse and NHP liver

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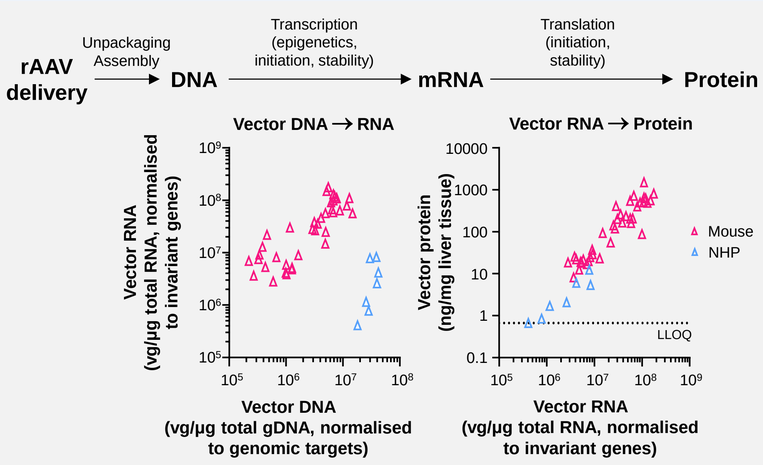
INTRODUCTION

- Understanding the mechanism(s) of variability of transgene expression within and between species is important for improved prediction and translation of recombinant adeno-associated virus (rAAV) gene therapy into the clinic
- In mouse and non-human primate (NHP) models, variability in vector transcription (DNA to RNA) is greater than in vector translation (RNA to protein), and transcriptional efficiency (expressed as RNA/DNA) is lower in NHP than in mouse (**Figure 1**)
  - Remarkably, transcriptional efficiency varies by a factor >500 between these species

Figure 1. Differences in transcription lead to variability in transgene protein expression across species



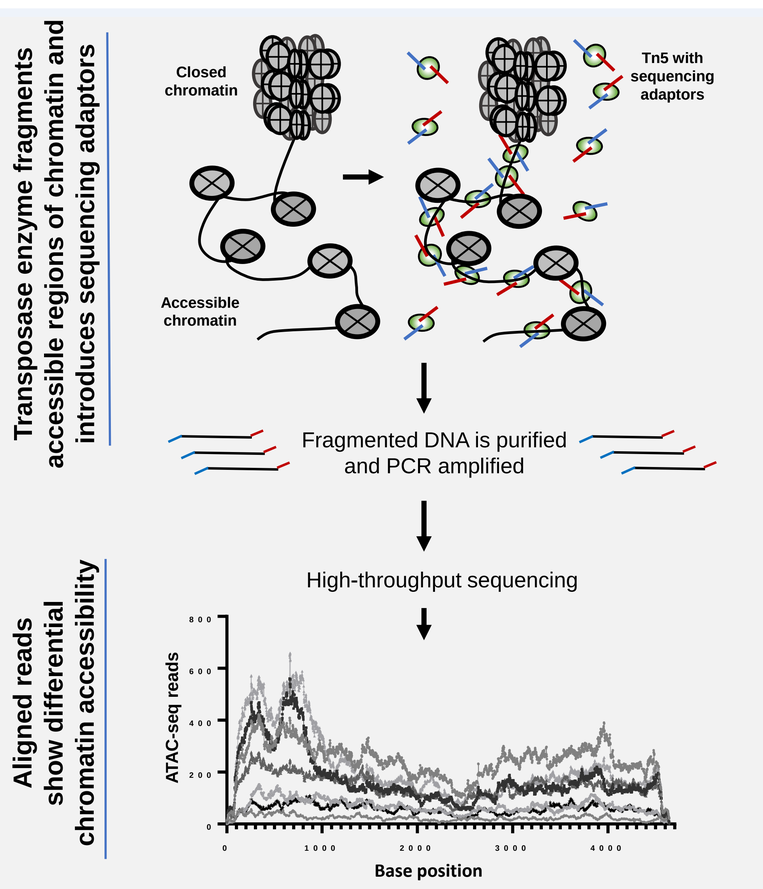
Vector 1 mouse data are from weeks 12, 24, and 52 post-dose; NHP data are from week 4 post-dose. gDNA, genomic DNA; mRNA, messenger RNA; NHP, non-human primate; rAAV, recombinant adeno-associated virus; vg, vector genome.

- Chromatin structure is regulated by many epigenetic modifications that influence transcriptional activity,[1] and rAAV vector DNA forms a chromosome-like structure with a nucleosome associated pattern[2]
- We investigated chromatin accessibility of rAAV vector genomes as a possible mechanism for variability of RNA production and transcriptional efficiency

METHODS

- Mice and NHPs were administered one of three different vector constructs, with liver-selective promoters delivered via rAAV serotype 5 (rAAV5) capsids at doses ranging from $2x10^{13}$ to $2x10^{14}$ vector genome/kg. Liver tissue samples were collected between weeks 1–52 after dosing
- Vector DNA and RNA levels were quantified using droplet digital polymerase chain reaction (ddPCR)
- Assay for transposase accessible chromatin sequencing (ATAC-seq) was performed on isolated nuclei and reads were aligned to the vector genome to evaluate the relationship between chromatinised vector and transgene expression (**Figure 2**)[3]

Figure 2. ATAC-seq method for epigenetic profiling identifies genomic loci of accessible chromatin



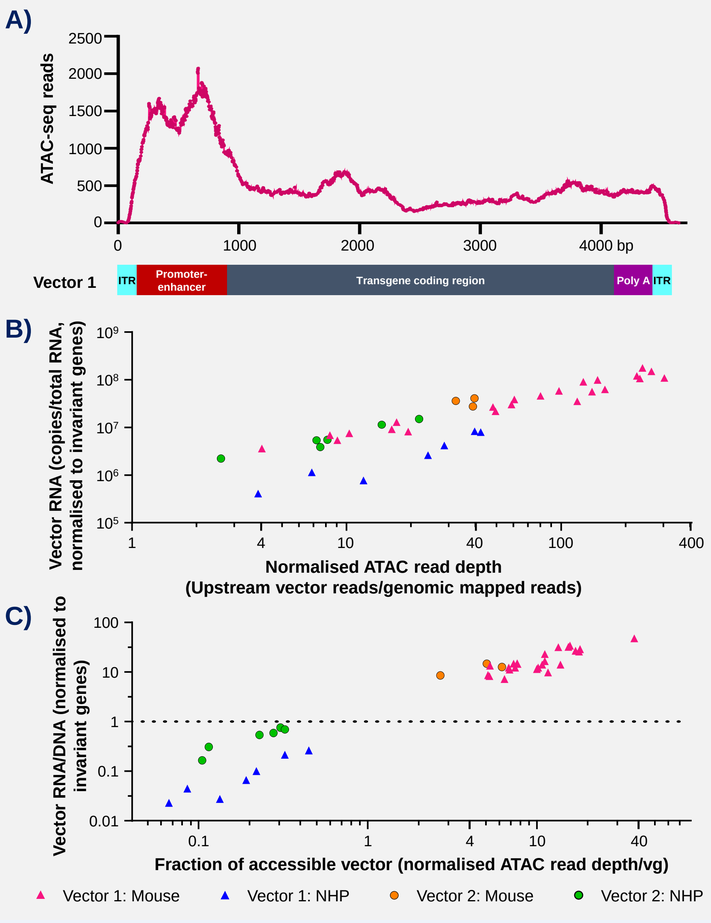
ATAC-seq read traces are from individual NHPs dosed with vector 1. ATAC-seq, assay for transposase accessible chromatin sequencing; NHP, non-human primate; PCR, polymerase chain reaction; Tn5, transposase 5.

- Chromatin immunoprecipitation sequencing (ChIP-seq), a high-throughput method for assessing protein-DNA interactions, was performed on samples. Sequencing reads were aligned to the vector genome to identify active and repressive histone modifications that correspond to chromatin accessibility

RESULTS

- ATAC-seq read depth aligned to the vector genome was enriched in the upstream enhancer-promoter region, indicating greater vector DNA accessibility (**Figure 3A**)
- Vector RNA production was correlated to total accessible vector DNA, suggesting the amount of open chromatin in the upstream region of the vector genome explains much of the intra-species variability in RNA expression (**Figure 3B**)
- Transcriptional efficiency, or RNA produced per DNA molecule, was positively correlated to the fraction of total vector DNA that is accessible in the upstream region, suggesting that the fraction of accessible vector DNA explains much of the inter-species variability in RNA/DNA (**Figure 3C**)
  - The total vector DNA was higher in NHPs than mice, but the amount of accessible chromatin was lower in NHPs than mice; increased accessibility leads to higher transcriptional activity

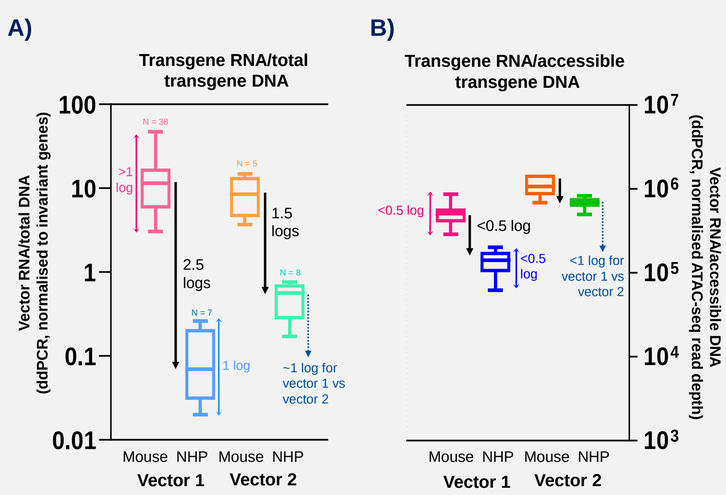
Figure 3. Accessible chromatin and RNA expression. A) Representative sample trace of ATAC-seq read depth across the vector genome. B) Total vector RNA production and total ATAC-seq reads in the promoter-enhancer region. C) Transcriptional efficiency and fraction of the vector genome that is accessible in the promoter-enhancer region



In panel A, data are representative from a single mouse sampled at 12 weeks post-dose.
Vector 1 mouse data are from weeks 12, 24, and 52 post-dose; NHP data are from week 4 post-dose. Vector 2 mouse data are from week 4 post-dose; NHP data are from week 9 post-dose.
ATAC, assay for transposase accessible chromatin; ATAC-seq, ATAC sequencing; NHP, non-human primate; ITR, inverted terminal repeat; poly A, polyadenylation sequence; vg, vector genome.

- For two different rAAV5 vector constructs, transcriptional efficiency was lower in NHPs than in mice, as measured by ratio of total RNA/DNA (**Figure 4A**)
- We assessed the efficiency of transcription from open-vector DNA by normalising vector RNA expression by ATAC-seq read coverage (**Figure 4B**)
  - Differential vector DNA accessibility accounted for much of the difference in transcriptional efficiency across species and constructs

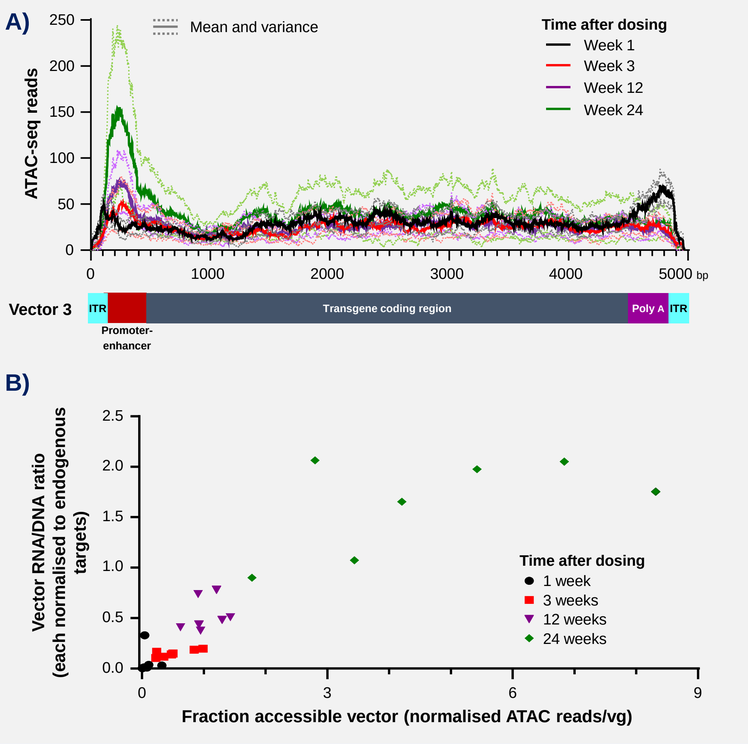
Figure 4. Transcriptional efficiency of rAAV5 vectors, as measured by A) RNA/DNA ratio and B) RNA/accessible DNA ratio



Vector 1 mouse data are from weeks 12, 24, and 52 post-dose; NHP data are from week 4 post-dose. Vector 2 mouse data are from week 4 post-dose; NHP data are from week 9 post-dose. Whiskers represent the minimum and maximum; boxes represent the 25th percentile, median, and 75th percentile.
ATAC-seq, assay for transposase accessible chromatin sequencing; ddPCR, droplet digital PCR; NHP, non-human primate; ddPCR, droplet digital polymerase chain reaction; rAAV5, recombinant adeno-associated virus serotype 5.

- Differences in chromatin accessibility were established early, with peak accessibility observed at week 24 (**Figure 5A**)
  - The fraction of accessible chromatin regulated transcriptional efficiency over time (**Figure 5B**)

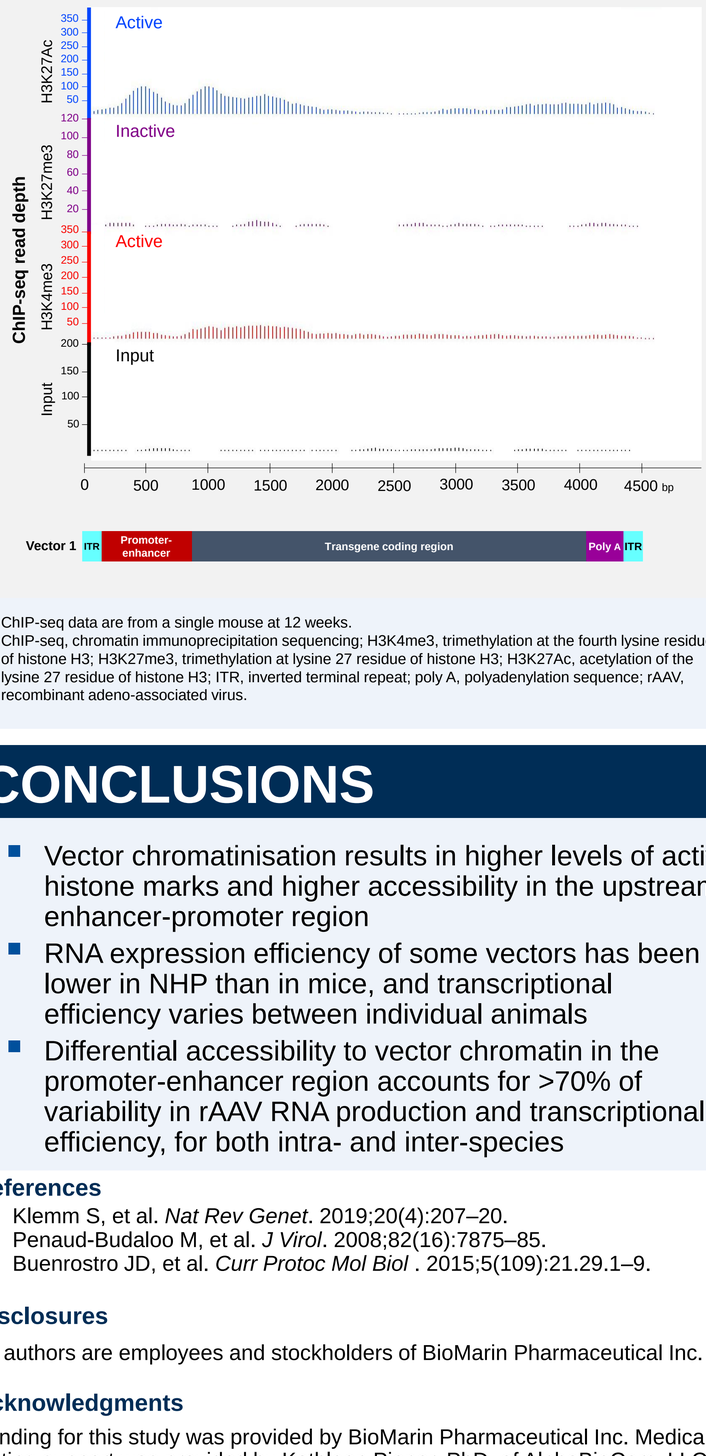
Figure 5. Changes in vector chromatin accessibility over time. A) Chromatin accessibility traces along the vector genome. B) Transcriptional efficiency and fraction of accessible vector chromatin



All data are from mice administered vector 3. In panel A, solid lines represent the mean ATAC-seq read depth and dashed lines represent the variance around the mean.
ATAC, assay for transposase accessible chromatin; ATAC-seq, ATAC sequencing; ITR, inverted terminal repeat; poly A, polyadenylation sequence; vg, vector genome.

- Preliminary ChIP-seq data identified active and repressive histone modifications that correspond to the chromatin accessibility observed in the promoter-enhancer region (**Figure 6**)
  - Additional analyses with a larger dataset are in progress

Figure 6. Active histone marks along the rAAV vector genome are enriched in the enhancer-promoter region



ChIP-seq data are from a single mouse at 12 weeks.
ChIP-seq, chromatin immunoprecipitation sequencing; H3K4me3, trimethylation at the fourth lysine residue of histone H3; H3K27me3, trimethylation at lysine 27 residue of histone H3; H3K27Ac, acetylation of the lysine 27 residue of histone H3; ITR, inverted terminal repeat; poly A, polyadenylation sequence; rAAV, recombinant adeno-associated virus.

CONCLUSIONS

- Vector chromatinisation results in higher levels of active histone marks and higher accessibility in the upstream enhancer-promoter region
- RNA expression efficiency of some vectors has been lower in NHP than in mice, and transcriptional efficiency varies between individual animals
- Differential accessibility to vector chromatin in the promoter-enhancer region accounts for >70% of variability in rAAV RNA production and transcriptional efficiency, for both intra- and inter-species

References

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Disclosures

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