REPURPOSING SINGLE-CELL RNA SEQ DATA TO QUANTIFY HIV SPLICING DURING LATENCY REVERSAL

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BACKGROUND

Causes of HIV-1 latency are incompletely understood. 10x Genomics single cell RNA seq has been used to quantify changes in cellular gene expression as cells transition out of latency, but little is known about changes in HIV-1 transcripts during latency reversal. One theory is that low levels of Tat or Rev transcripts induce latency. If Tat transcripts were insufficient to produce adequate Tat protein, transcription would tend to abort. Insufficient Rev would result in a functional latency by preventing export of unspliced and partially spliced HIV-1 mRNAs. If a downward spiral of Tat or Rev transcripts were crucial in establishing and maintaining latency, we would expect latent cells to be less likely to carry out the splicing events required to produce Tat or Rev transcripts.

METHODS

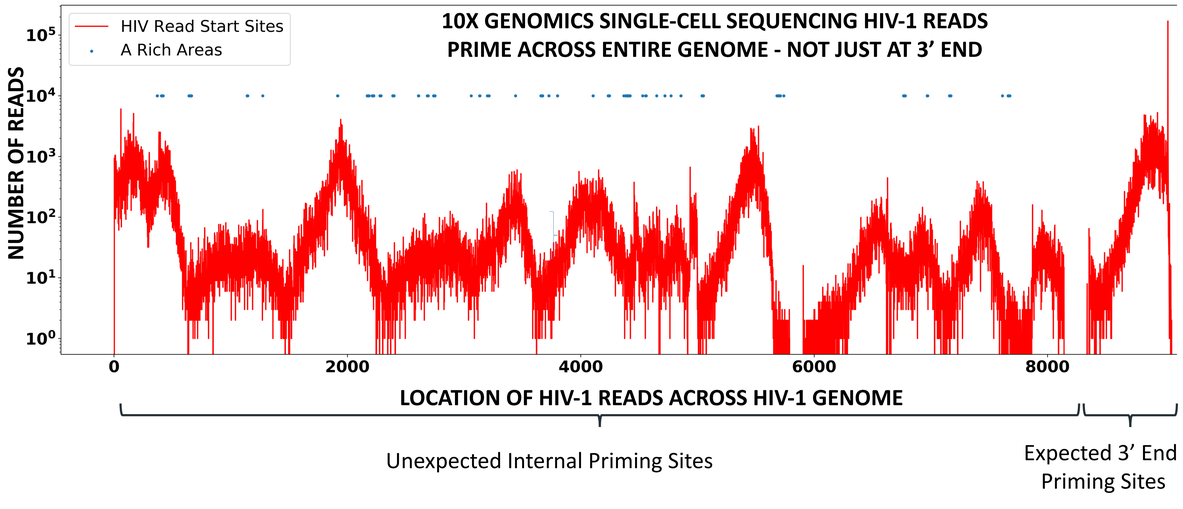
Our analysis used 10x Genomics single cell RNA seq data from a primary cell model of HIV-1 latency. CD4+ T cells were infected with a GFP expressing HIV-1 clone, allowing identification and selection of transcriptionally active cells. Over time these confirmed infected cells tended to become quiescent/latent and expressed a wide range of HIV-1 transcript levels at the time of sequencing.

In 10x Genomics single cell seq, each poly-A transcript is tagged with a barcode unique to each cell and an ID tag unique to each transcript. These tags are attached to a poly-T primer which targets only mRNA 3' ends - and such 3' end reads are useless in identifying specific HIV-1 transcript types or splicing activity. However, we found that 68% of HIV-1 transcripts were unexpectedly primed across the entire A-rich HIV-1 genome, allowing identification of specific transcript types based on location and splicing junctions.

Unintended priming of HIV-1 transcripts in the production of 10x Genomics single cell sequencing libraries is a rich source of additional information about HIV transcript types, splicing activity, and changes in relative abundance of specific transcripts across a range of HIV-1 transcriptional activation states.

RESULTS

Internally primed HIV-1 reads in single cell seq data allowed precise identification and quantification of expressed viral sequences across the genome. Additionally, the relative level of HIV-1 expression in a cell could be inferred as a ratio of HIV-1 transcripts to cellular transcripts. Although any one individual cell lacks sufficient HIV reads to determine expression patterns, we binned cells into categories based on the percentage of HIV-1 transcripts in the cell. We observed clear patterns of HIV transcript changes as cells went from low to high HIV-1 expression: unspliced transcripts increased uniformly and spliced transcripts (including Tat specifically) decreased. Rev transcripts also decreased (data not shown). Thus the HIV-1 transcription program during latency reversal closely follows the program seen during acute infection. These changes were validated using a second 10x Genomics single cell data set from an N6 Jurkat latent cell line chemically activated with AZD + iBET.

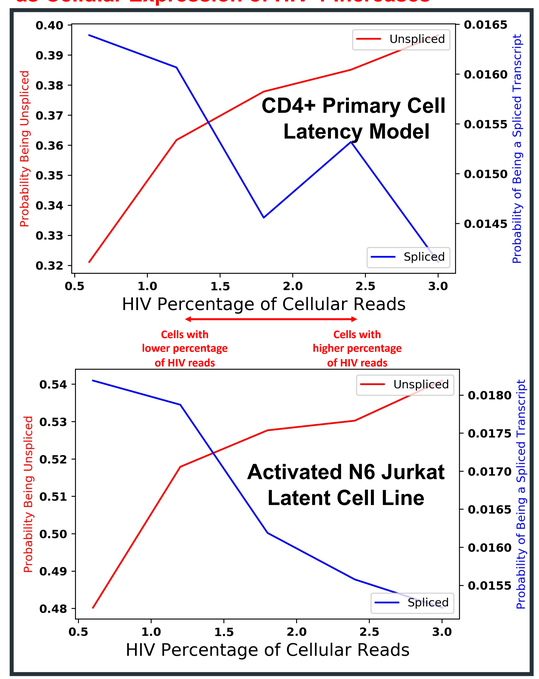


CONCLUSIONS

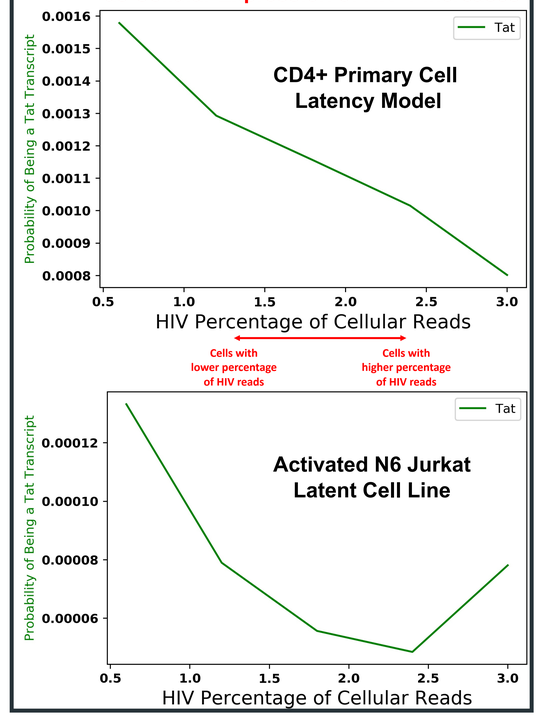
We saw consistency in the types of HIV-1 transcripts identifiable in 10x Genomics single cell seq data from two HIV-1 latency models. Transcripts are more likely to splice when the proportion of HIV-1 transcripts is low and more likely to remain unspliced or partially spliced as the proportion of HIV-1 increases. Consistent with our understanding of the roles of Tat and Rev in lytic infection, our results show that a low cellular level of HIV-1 transcripts increases the probability that any given HIV-1 transcript will be a Tat or Rev transcript, which does not favor the theory of a Tat/Rev induced latency.

Our findings indicate that unexpected priming across the HIV-1 genome enables detailed quantification of specific HIV-1 transcripts and yields additional insights from existing HIV-1 single cell RNA seq data sets.

Unspliced Transcripts Increase/Spliced Transcripts Decrease as Cellular Expression of HIV-1 Increases



Tat Transcripts Decrease as Cellular Expression of HIV-1 Increases



GOT DATA? – We're interested in testing our pipeline on additional HIV-1 single cell data sets
Contact: aemery@email.unc.edu