The HIV-1 Protein ASP Promotes Viral Replication and Is Associated with Faster Disease Progression

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BACKGROUND: The HIV-1 antisense gene *asp* overlaps *env* at the SU/TM boundary, encodes a protein (ASP) of ~189aa. *Asp* is present exclusively in pandemic group-M HIV-1 strains. Thus, we studied the evolutionary mechanisms that created *asp*. We reported that ASP is present on the surface of HIV-1 virions and shows nuclear and cytoplasmic distribution during viral latency and replication, respectively. We also investigated the impact of ASP on HIV-1 entry and replication, and on host cell functions.

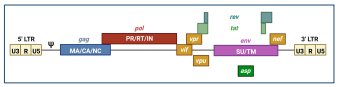


Fig. 1. Genome of HIV-1 strains of group M showing the *asp* gene that overlaps *env*.

METHODS: *In silico* methods for the evolutionary processes that created *asp*. *In vitro* fusion and replication assay of WT vs. ASP-deficient HIV-1. MS and co-IP for identification of ASP binding partners. Nuclear import, DNA repair, and mitochondrial factor expression for functional validation.

RESULTS

1. Creation of the HIV-1 asp gene.

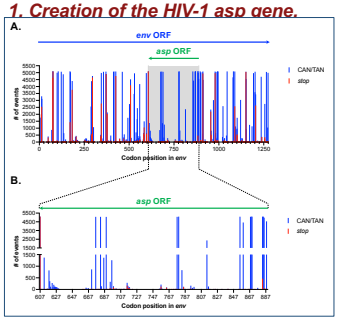


Fig. 2. CAN/TAN codons in *env* are hotspots for *stop* codons in the –2 antisense frame. Different frequency of *stops* outside *vs* inside the *env/asp* overlap. Distribution of CAN/TAN codons in *env* (blue peaks) and *stops* in the –2 antisense frame (red peaks) in the *env* gene (**A**) and within the *asp* ORF (**B**).

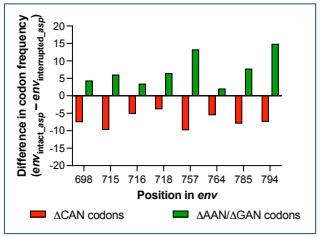


Fig. 3. Codon positions with statistically significant decrease in the content of CAN codons (red columns) and a significant increase in the content of AAN/GAN codons (green columns) in *env* overlapping an intact *vs* truncated asp ORF.

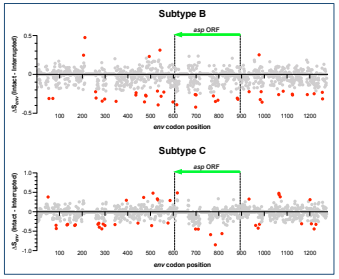


Fig. 4. Different entropy at each codon position of *env* (ΔS_{env}) between sequences overlapping an intact *vs* an interrupted *asp* ORF. Red circles are positions with ΔS_{env} value significantly different than the mean value along the entire *env* sequence.

2. Impact of ASP on HIV-1 entry, replication, and disease progression.

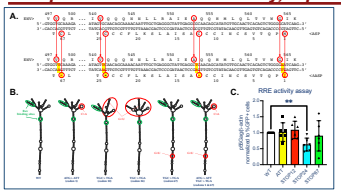


Fig. 5. Nucleotide substitutions that eliminate the *start* codon or introduce early *stops* in *asp* (**A**) do not affect the predicted structure (**B**) or function of RRE that partially overlaps *asp* (**C**).

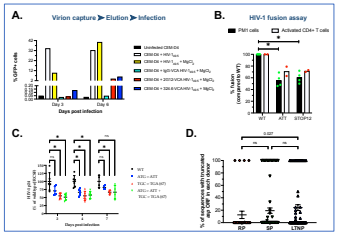


Fig. 6. Anti-ASP antibodies (324.6) capture infectious HIV-1 virions (**A**). Reduced fusion (**B**) and replication (**C**) of ASP-knockout strains compared to WT. **D:** Higher frequency of intact *asp* ORF in rapid progressors (◆) *vs* slow progressors (▼) and long-term non progressors (▲).

3. The ASP interactome: DNA repair and mitochondrial function.

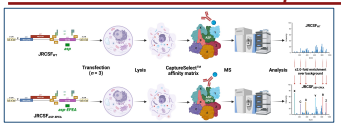
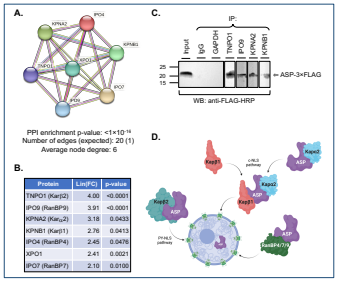


Fig. 7. Experimental approach for the identification of ASP-binding partners by MS.



Protein	Lin(FC)	p-value
TNPO1 (Karβ2)	4.00	<0.0001
IPO9 (RanBP9)	3.91	<0.0001
KPNA2 (Karα2)	3.18	0.0430
KPNB1 (Karβ1)	2.76	0.0413
IPO4 (RanBP4)	2.45	0.0476
XPO1	2.41	0.0021
IPO7 (RanBP7)	2.10	0.0106

Fig. 8. Network of nuclear import/export proteins interacting with ASP (**A**). Enrichment and p-value of nuclear import/export factors interacting with ASP (**B**). Validation of ASP binding to four nuclear import factors (**C**). Pathways of nuclear import via Karβ1/Karα2, Karβ2 (TNPO1), and RanBP4/7/9 (**D**).

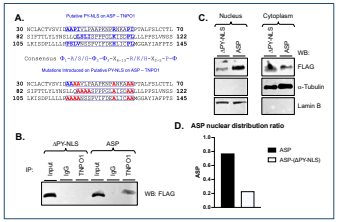


Fig. 9. A: ASP motifs matching the Karβ2 (TNPO1) consensus sequence and generation of an ASP variant with mutations in the three motifs. The ASP variant fails to interact with TNPO1/Karβ2 (**B**) and shows reduced nuclear accumulation (**C** and **D**).

REFERENCES

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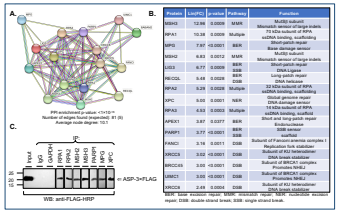


Fig. 10. Network of DNA damage repair factors binding ASP (**A**). Enrichment, p-value, and DNA repair pathway of each ASP-binding protein (**B**). Validation of ASP binding to seven DNA repair factors (**C**).

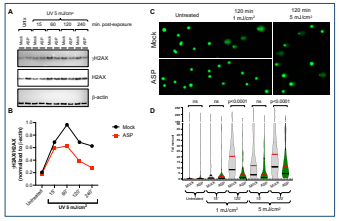


Fig. 11. ASP expression reduces peak levels and accelerates return to baseline levels of phosphorylated H2AX (γH2AX) (**A** and **B**), and it promotes faster resolution of UV-induced DNA damage (comet assay, **C** and **D**).

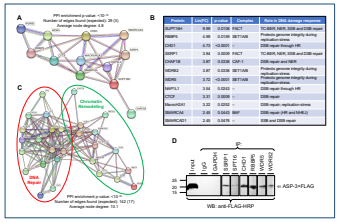


Fig. 12. Network of chromatin factors involved in DNA repair that bind ASP (**A**) and association with repair factors in Fig. 10 (**C**). Enrichment, p-value, and role in DNA repair of ASP-binding factors (**B**). Validation of ASP binding to six chromatin binding factors (**D**).

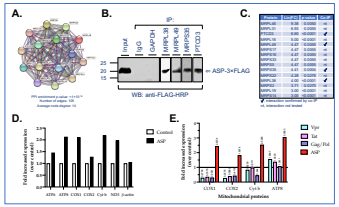


Fig. 13. Mitochondrial ribosomal proteins that bind ASP (**A**). Co-IP validation (**B**). Enrichment and p-value of mitochondrial ribosomal protein binding ASP (**C**). ASP increases expression of mtDNA-encoded factors involved in ATP synthesis (**D** and **E**).

- The HIV-1 antisense gene *asp* is unique to pandemic group-M HIV-1 strains, and it is absent in all other primate lentiviruses
- Creation of *asp* involved nucleotide substitutions that eliminated premature *stops* in *asp* but did not alter *env* on the opposite strand
- Conservation of an intact *asp* restricts the genetic diversity of *env*
- ASP expression contributes to HIV-1 entry and replication *in vitro* and is associated with faster disease progression in PWH
- Analysis of the ASP interactome reveals that ASP interacts with host DNA repair factors and promotes faster resolution of DNA damage
- ASP interacts with mitochondrial ribosomal proteins and increases the expression of mtDNA-encoded enzymes involved in ATP synthesis
- Our results suggest that creation and conservation of *asp* brought a net selective advantage to HIV-1 strains of group M, or it would have likely been lost to random genetic drift

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