New tools to study the expression of the Antisense Protein gene in the proviral DNA context

Yong Xiao, Caroline Toudic
and Benoit Barbeau
Université du Québec à Montréal
Réseau intersectoriel de recherche en santé de l'Université du Québec

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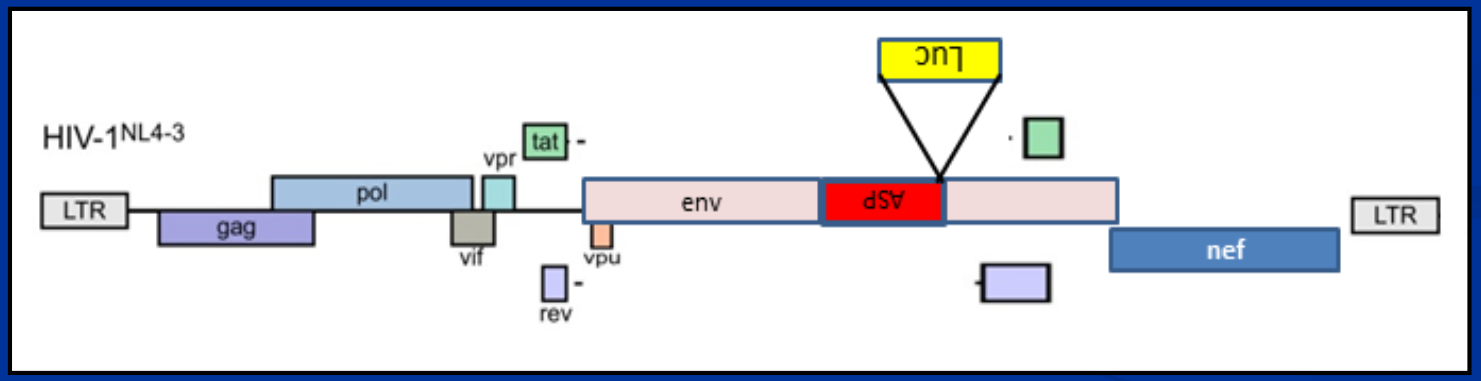
The HIV-1 Antisense Protein (ASP) gene

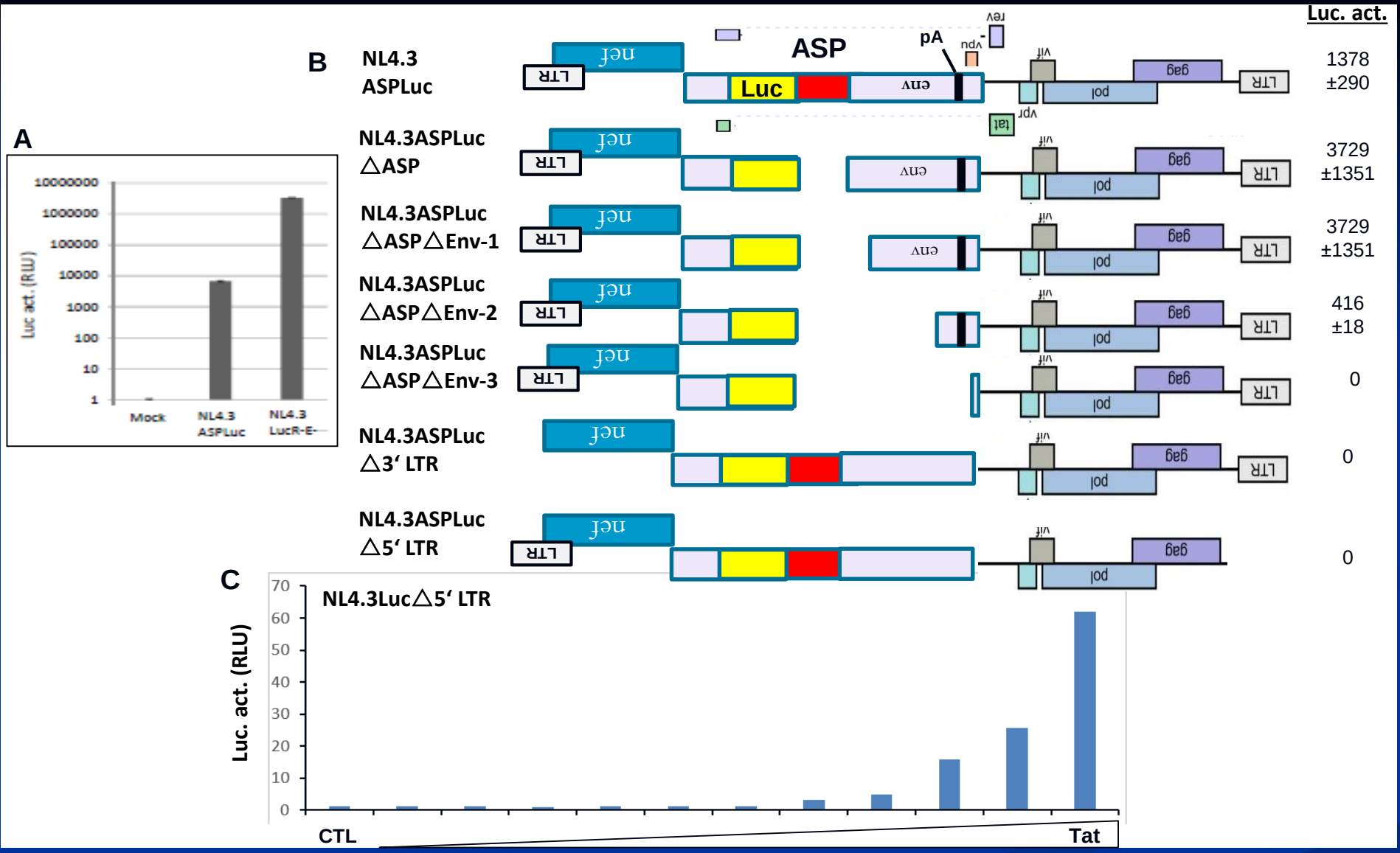
- First identified *in silico* and supported by the identification of antisense transcripts driven by the 3' LTR (Miller, 1988; Michael et al., 1994; Landry et al., 2007; Saayman et al., 2014; Kobayashi-Ishihara et al., 2018)
- Specific CTL activity and antibody response detected in infected individuals (Vanhee-Brossollet et al., 1995; Bansai et al., 2015; Bet et al., 2015; Savoret et al., 2020)
- Coding capacity of ASP ORF supported by its presence in more dominant HIV-1 clades (Cassan et al., 2016)
- Detection in overexpression condition with various clade representatives (but not in proviral DNA) (Clerc et al., 2011; Laverdure et al., 2012; Torresilla et al., 2013; Liu et al., 2019)



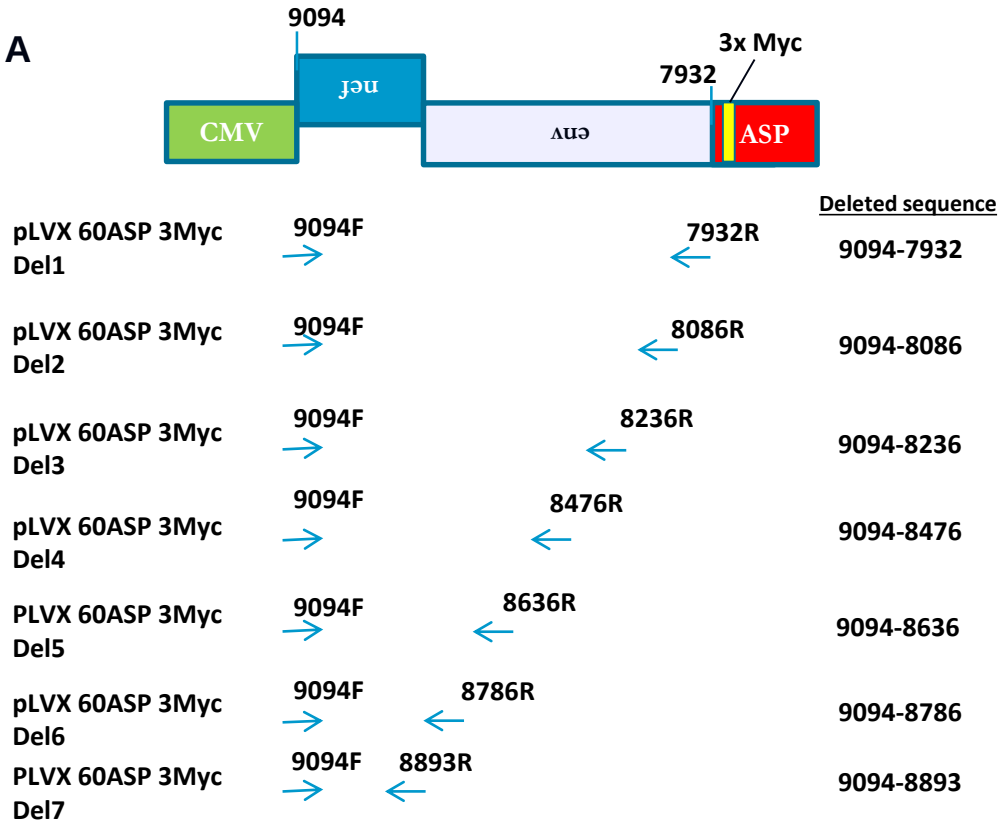
Objectives

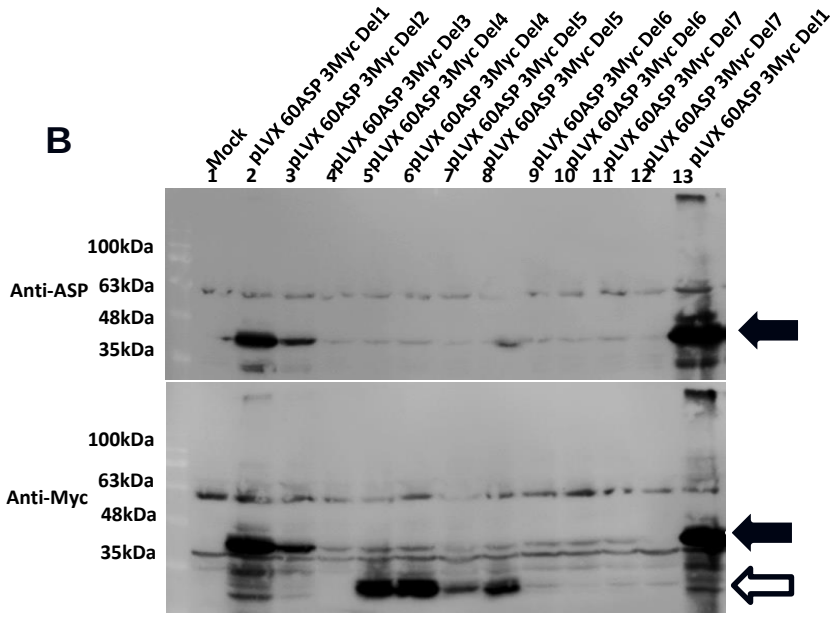
1-To confirm the encoding potential of antisense transcripts using proviral DNA constructs containing the luciferase gene inserted in the position of the ASP ORF

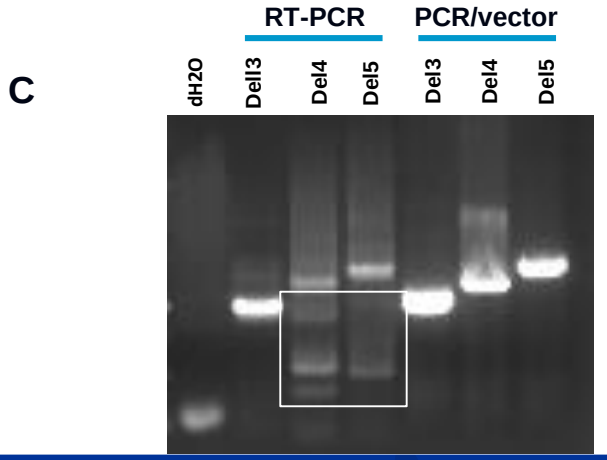
2- To detect the ASP protein in the provirus context by the use of an alternative promoter and the addition of tags



The expression of antisense transcript-dependent luciferase in the proviral DNA relies on LTRs and the 3'UTR and is Tat-inducible. A. 293T cells were transfected with NL4.3ASPLuc (antisense) or NL4.3LucR-E- (sense) (vs. mock). **B-C**. 293T cells were transfected with NL4.3ASPLuc or deleted versions. In **C**, NL4.3ASPLuc△5' LTR was co-transfected with increasing amounts of a Tat expression vector (vs. empty vector: CTL). Luciferase activities were measured in three independently transfected samples (except for panel **C**). Results argue that antisense transcripts can be translated and that luciferase expression is detectable when postioned next to the ASP ATG. Data also demonstrate the importance of both LTRs and a presumed polyA (pA) signal and for induced expression of the 5' LTR-deleted construct in the presence of Tat.

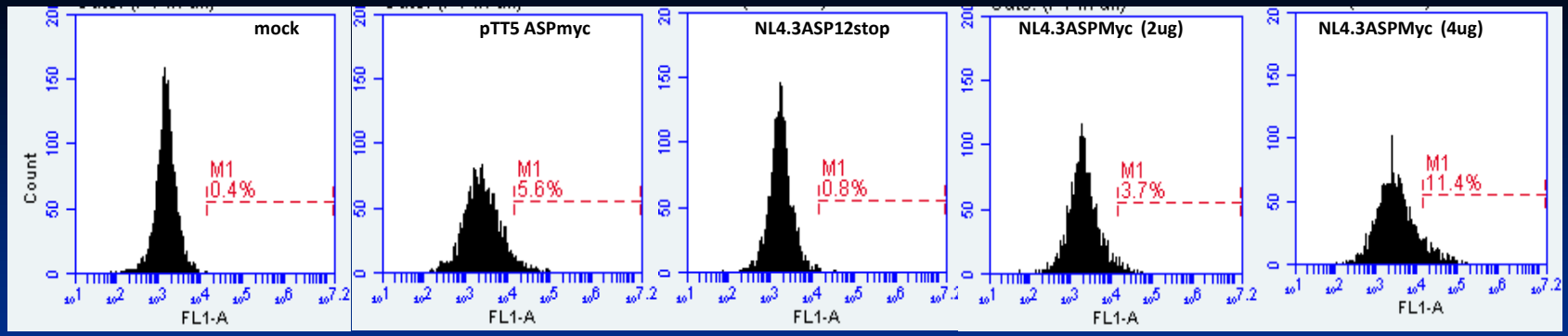




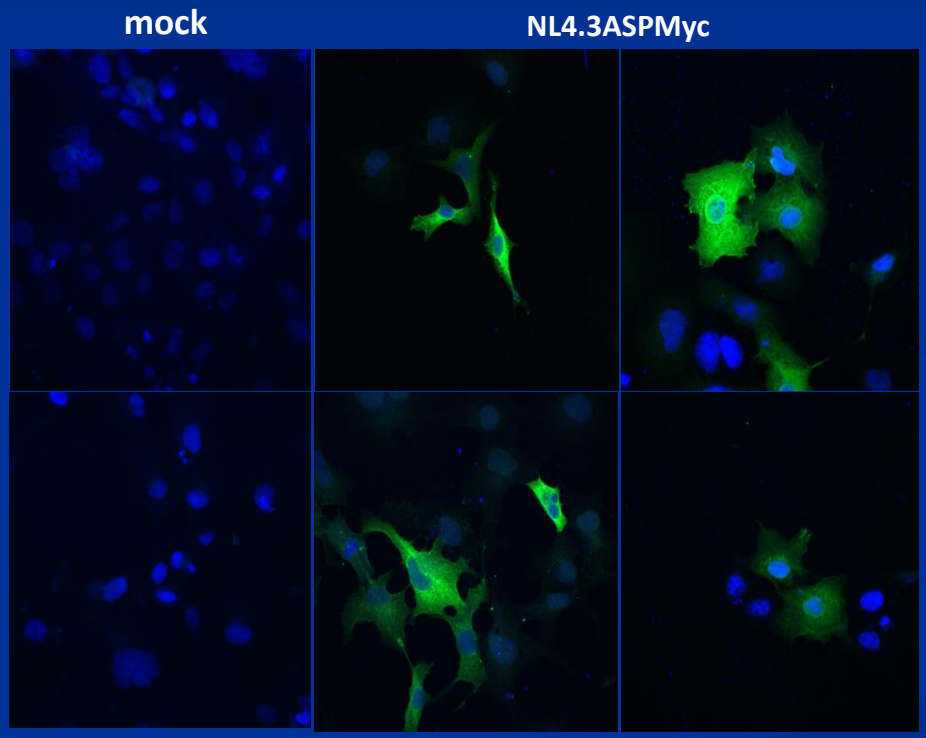


CMV-driven expression of proviral DNA-based ASP is detectable and highlight potential spliced transcripts. The 3' end of NL4.3 containing the ASP ORF with a Myc tag at amino acid 60 and the corresponding upstream sequence lacking the 3' LTR (nt 9094-7932) was inserted in an antisense orientation downstream of a CMV promoter. Versions of this construct with various deletions in the upstream sequence (Del1 to Del7) (**A**) were transfected (some in duplicate) in 293T followed by WB analyses with anti-Myc and anti-ASP antibodies (**B**). Filled arrows: ASP; empty arrow: new isoform. **C**. 293T transfected with Del3, Del4 and Del5 constructs were analysed by RT-PCR using primer 9094F (**A**) and a ASP-specific reverse primer. Similar primers were used for PCR testing of plasmid DNA (empty rectangle presents spliced signals). Results demonstrate that a strong promoter allows ASP detection by WB and that a region in the 5'UTR of the transcript leads to alternative splicing with the synthesis of another isoform.

A



B



Detection of proviral DNA-expressed tagged ASP is possible by flow cytometry and confocal microscopy. pTT5-expressing ASP (CMV-driven ASP cDNA), NL4.3-expressing Myc-tagged ASP (NL4.3ASPMyc) (2-4 ug) or NL4.3 containing a stop codon at position 12 in the ASP ORF were transfected in 293T cells and analysed by flow cytometry (A) or confocal microscopy (B) with anti-ASP antibodies. Results demonstrate that ASP expressed from proviral DNA can be detected using these approaches.