KU LEUVEN

The role of HIV nuclear import in integration site selection and the transcriptional state of the integrated provirus.

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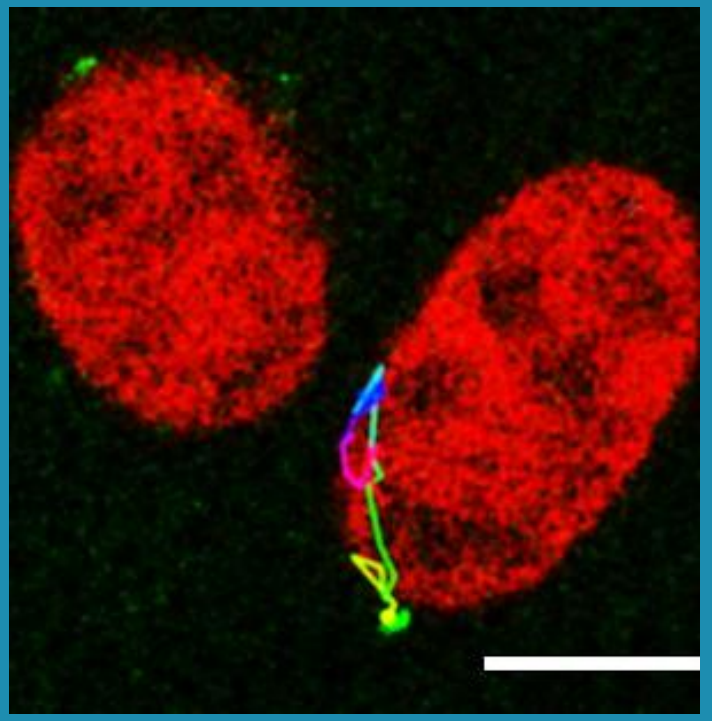


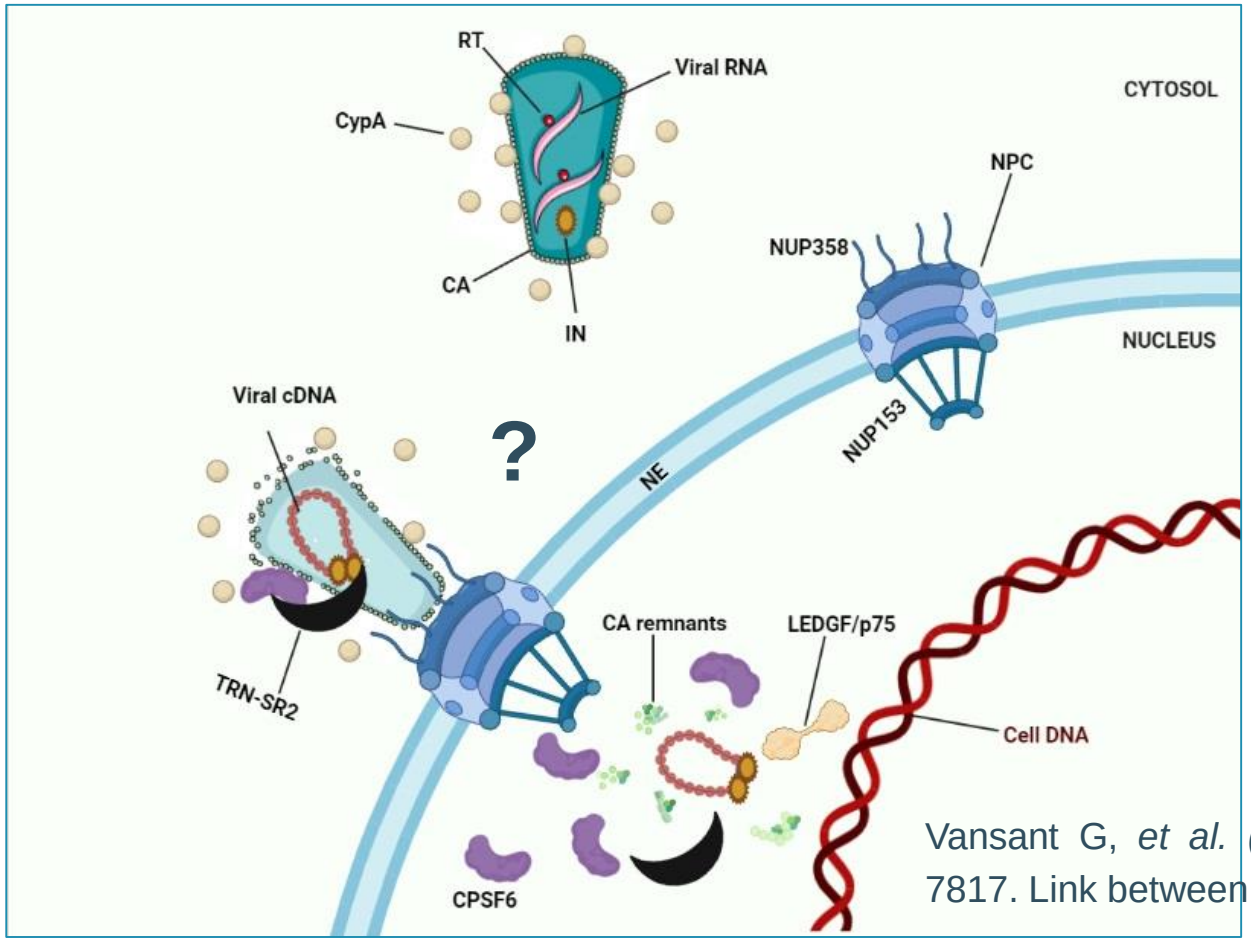
Rationale

- Integration site selection and the chromatin landscape of the integration site determine the transcriptional state of the provirus in the reservoir cells [1, 2].
- Recently, it was reported that intact proviral sequences from elite controllers are enriched in repressive chromatin marks, providing a clinical correlate of this paradigm [3].
- **Since nuclear import as well determines integration sites [4], we will investigate in this project the role of nuclear import on integration site selection and the resulting transcriptional state.**
- This basic research will increase our insight in the mechanisms underlying HIV persistence and open perspectives for future (functional) cure strategies

References:

1. Vranckx, L. S., *et al*. (2016). *EBioMedicine*, 8, 248-264.
2. Vansant G, *et al. (2020)*, *Nucleic Acids Res.* 2020 48(14):7801-7817.
3. Jiang C., *et al.,* (2020) *Nature* 585(7824):261-267.
4. Ocwieja, K. E., *et al*. (2011). *PLoS pathogens*, 7(3), e1001313.

Model for nuclear import, integration and transcription



Vansant G, *et al. (2020)*, *Nucleic Acids Res.* 2020 48(14):7801-7817. Link between integration site and transcription.

Work packages

WP1 To study the role of host factors in nuclear import: TRN-SR2,TNPO1 and CPSF6.

- **We hypothesize that host transportins play specific roles during nuclear import.** Further studies are needed to fully corroborate their roles during HIV infection. We have identified TRN-SR2 as a binding partner of HIV integrase whereas TNPO1 has recently been described to interact with HIV capsid. Using cells depleted for either factor, single virus imaging technologies will be used to study the different parameters of HIV PICs during nuclear import and integration.

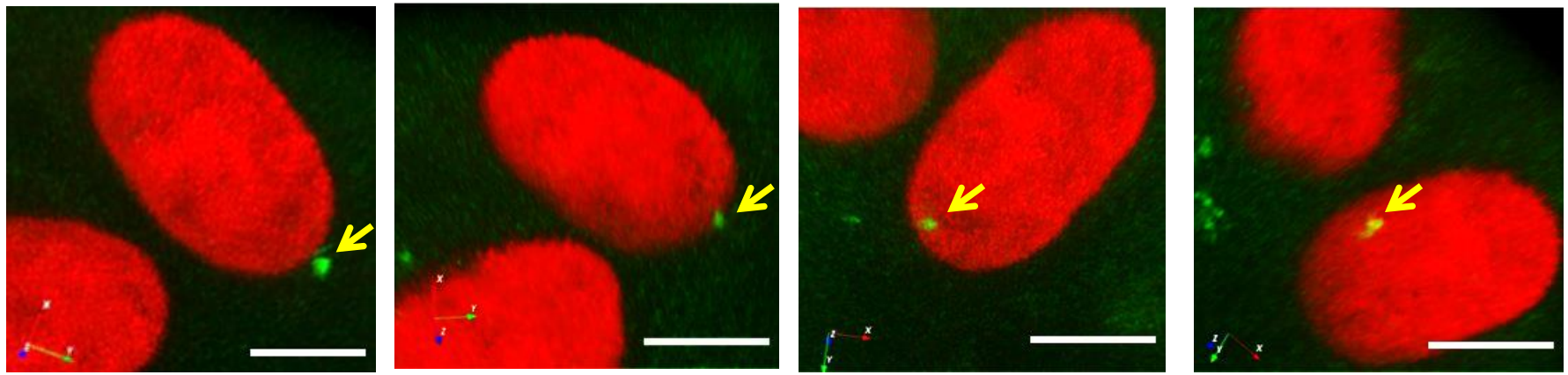
WP2 to determine the impact of integration sites on HIV transcription and hence HIV persistence.

- **We hypothesize that nuclear import and integration affect integration sites and RNA transcription.** We plan to analyze viral DNA/RNA using bDNA imaging after depletion of TRN-SR2 or TNPO1 to reduce nuclear import. This study will reveal the relative contribution of nuclear import in integration site selection and is impact on transcription

Technologies

- I Single virus live imaging

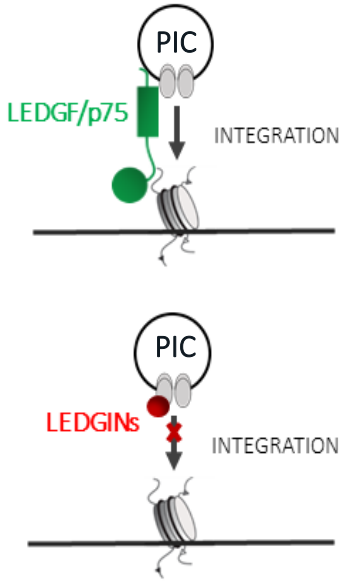
Tracking the nuclear import of IN-eGFP viral particles in living cell

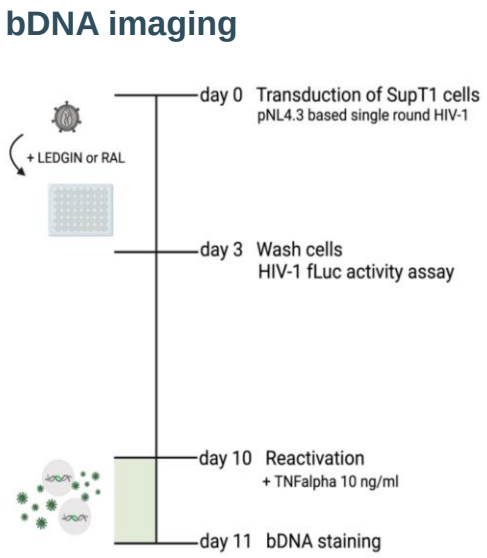


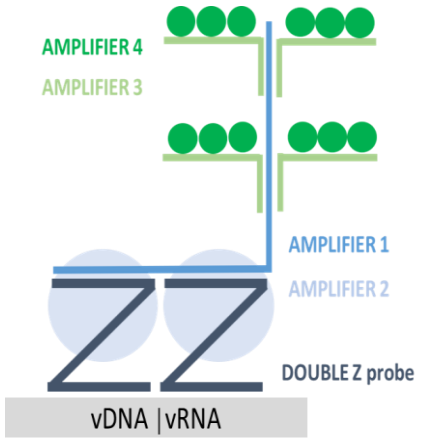
Time post-infection: 0:50:20 0:52:20 0:56:20 0:58:20 Scale Bar: 10 um

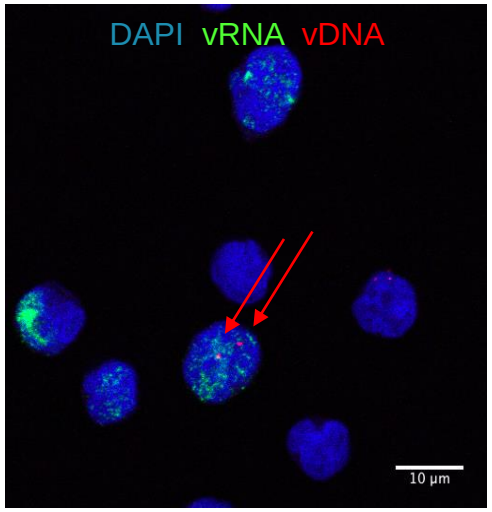
Technologies

- II bDNA imaging of virus RNA and DNA







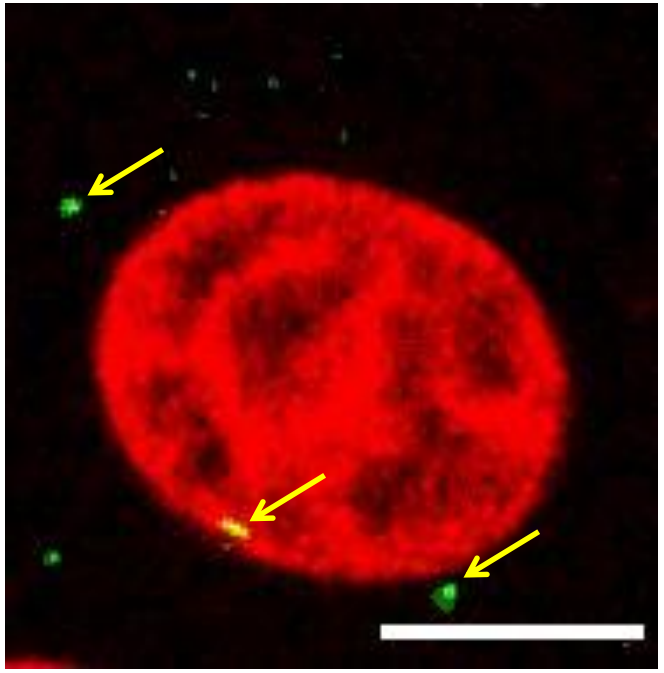


Work plan

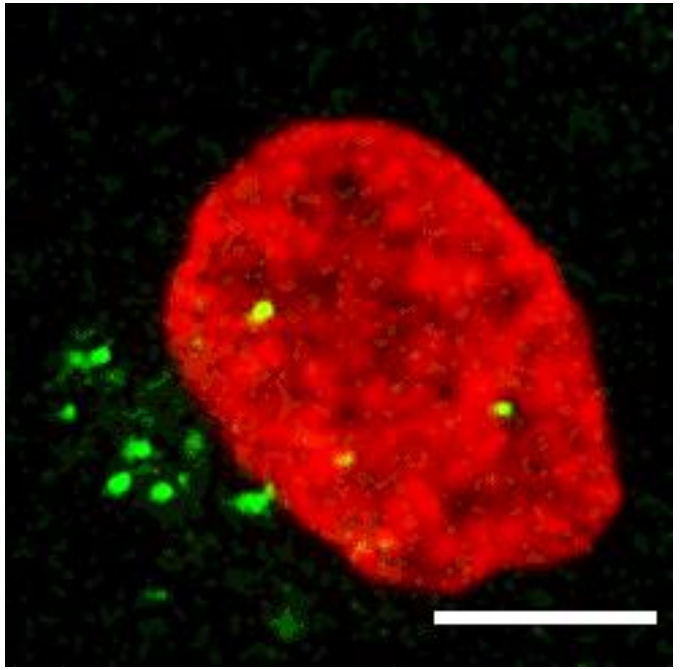
Tasks	Year 1				Year 2			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
WP1. The role of two host factors in nuclear import: TRN-SR2								
1.1. Import by TRN-SR2		D1.1						
1.2. Import by TNPO1				D1.2 P1				
WP2. Determination of the impact of integration sites on HIV transcription and HIV persistence.								
2.1. bDNA imaging TRN-SR2						D2.1		
2.2. bDNA imaging TNPO1								D2.2 P2

Legend: **D** deliverables **P** publication submission

RESULTS: nuclear import is reduced in CPSF6 depleted cells



HeLa WT



CPSF6 KD

Images 2 hours post infection

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