Capsid-CPSF6 interaction: Master regulator of nuclear HIV-1 positioning and integration

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Abstract HIV-1 integration favors active chromatin, which is primarily mediated through interactions between the viral capsid and integrase proteins with host factors cleavage and polyadenylation specificity factor 6 (CPSF6) and lens epithelium-derived growth factor/p75, respectively. Previously published image-based studies had suggested that HIV-1 prefers to integrate into chromatin that associates spatially with the nuclear periphery. Here, we re-evaluated previously reported HIV-1 nuclear distance measures across studies and show that HIV-1 prefers peri-nuclear and mid-nuclear zones similarly, with a common preference between studies mapping to the boundary between these two radial areas. We also discuss emerging roles for the capsid-CPSF6 interaction in facilitating HIV-1 pre-integration complex nuclear import and subsequent intranuclear trafficking to preferred sites of viral DNA integration.
Keywords: HIV nuclear trafficking; CPSF6; HIV integration; HIV capsid; HIV integrase; LEDGF/p75; nuclear import; pre-integration complex.

HIV-1, like all retroviruses, reverse transcribes its RNA genome into DNA and integrates the DNA copy into a host cell chromosome. After integration, retroviruses rely on host transcription machinery to produce viral proteins and genomic RNA, both of which co-assemble into nascent viral particles. The HIV-1 ribonucleoprotein complex, which consists of the RNA together with viral nucleocapsid proteins and replication enzymes reverse transcriptase and integrase (IN), is encased by a conical capsid shell composed of the viral capsid (CA) protein (reviewed in [1]).

After HIV-1 enters a susceptible target cell via lipid membrane fusion, reverse transcription happens within a subviral nucleoprotein complex called the Reverse Transcription Complex (RTC) [2]. CA protein within the RTC helps to protect the viral DNA against host defense mechanisms in the cytoplasm [3-5], and the viral capsid shell is gradually shed from the RTC as it is transported towards the nucleus (reviewed in [1, 6, 7]). Once the RTC becomes competent for integration, it is referred to as the pre-integration complex (PIC) [8] and some CA protein remains associated with the HIV-1 PIC after nuclear entry [9-13]. IN functions as part of the intasome nucleoprotein complex composed of an IN multimer and the ends of the linear viral reverse transcript (reviewed in [14]).

HIV-1 integration targeting

HIV-1 integration into cellular DNA is not random, with the virus favoring the interior regions of transcriptionally active genes residing in relatively gene-dense regions of chromatin [15]. HIV-1 integration targeting preference is largely driven by the interaction of two viral proteins, IN and CA, with respective cellular proteins lens epithelium-derived growth factor (LEDGF)/p75 and cleavage and polyadenylation specificity factor (CPSF) 6. Depletion of either of these two host factors results in significant reduction of integration into genes and gene-dense regions [16-21]. Although depleting either

LEDGF/p75 or CPSF6 reduces intragenic integration, the two factors influence HIV-1 integration in different ways. LEDGF/p75 depletion shifts intragenic integration towards the 5′ end regions of the genes, whereas CPSF6 depletion results in HIV-1 dramatically losing preference for integration near activating epigenetic marks and instead favoring gene-sparse regions. These results suggest that LEDGF/p75 primarily functions to position integration along the genes, whereas CPSF6 predominantly shields HIV-1 from integration into heterochromatin [21]. It was unclear until our recent publication [22] how these contrasting roles of CPSF6 and LEDGF/p75 in HIV-1 integration targeting influenced viral DNA localization inside the nucleus.

HIV-1 nuclear localization during acute infection

Different imaging techniques such as induced double stranded DNA breaks (SCIP for single-cell imaging of HIV-1 provirus) [23, 24], fluorescently labelled IN proteins [25-29], fluorescently labelled cyclophilin A protein that interacts with CA [3], immuno-DNA fluorescent in situ hybridization (FISH) [30], branch-chain DNA FISH [11], click chemistry [9], and stimulated emission depletion (STED) microscopy [10] have been used to track HIV-1 PICs inside the nucleus. Analyses of intranuclear position are facilitated by determining the relative radial distance of the imaged HIV-1 focus from the nuclear envelope (NE), and binning the results into three concentric nuclear zones of equal area [30, 31]. The most peripheral zone, peripheral nuclear (PN), has a width of 0.184 x r (nuclear radius); the mid-nuclear (MN) zone ranges from 0.184 x r to 0.422 x r; and the inner-most central nuclear (CN) zone has a width of 0.422 x r (Fig. 1A). A majority of prior imaging studies had indicated that HIV-1 preferentially integrates into active chromatin within the PN area [3, 23, 25-27, 29] proximal to the nuclear pore [30]. But re-evaluating the reported radial distance measures across studies shows that HIV-1 PICs and proviruses target PN and MN areas similarly, with a preference for the boundary between these two areas [22].

Although HIV-1 normally infects CD4+ cells such as T cells and macrophages, numerous microscopy studies utilized HeLa cells (7.4 µm average nuclear radius) due to their compatibility with imaging technologies. HIV-1 PICs located within 0.4 - 2.0 µm from the NE of HeLa cell nuclei, equating to 0.05 - 0.25 x r, mapping to the PN and MN areas [25]. A separate study reported that the average distance traveled by PICs into HeLa cell nuclei was 1.2-1.6 µm, equating to 0.16-0.22 x r, again representing the PN and MN areas [26]. Tracking the translocation of fluorescently labeled HIV-1 complexes confirmed 1.4 µm (or 0.19 x r) as the average distance travelled into HeLa cell nuclei [29], equating to the interface between the PN and MN areas. Another study tracking the nuclear progression of single HIV-1 particles [3] measured 1.8 µm as the average distance travelled by PICs in HeLa cell nuclei, which is 0.24 x r (MN area). Imaging HIV-1 proviruses using the SCIP technique, Di Primio et al. [23] reported that 55% of HIV-1 proviruses mapped within 1.5 µm from the NE of U2OS cell nuclei, which equates to 0.19 x r and thus is consistent with the PN-MN interface. In this same report, the authors saw that 62% of integrated proviruses mapped within 0.5 µm from the NE of CEMss T cell nuclei at 2 days post infection, placing the virus at 0.09 x r (PN). However, eleven days later, HIV-1 proviruses distributed randomly throughout the T cell nuclei [23]. While the majority of HIV-1 (NL4-3 strain) nuclear foci localized in the PN area of primary CD4+ T cells, the related HIV-1 (BRU) strain localized similarly to the PN and MN areas [30].

Visualizing HIV-1 proviruses by branched-DNA FISH technology, we initially reported preferential localization of HIV-1 PICs and proviruses to the PN area of primary CD4+ T cell nuclei [11]. However, by using radial cutoffs of 0.5 x r for CN and 0.5 x r to 0.75 x r for MN, we since realized our prior bin sizes were modestly unequal (the proper radial cutoffs for respective CN and MN boundaries are 0.58 x r and 0.82 x r; Fig. 1A) [30-32], which underrepresented the CN area by ~25% and inflated PN area by ~30% (Fig.

1A and 1B). The fractional PN localization that we had previously documented became equalized with other nuclear sections when datasets were reanalyzed using the corrected areas (Fig. 1C-D). In summary, imaging experiments do not strongly support preferential localization of HIV-1 in the nuclear periphery. We recently showed, using multiple orthologous approaches, that HIV-1 locates equally to the PN and MN areas, with some penetration into the CN area as well [22]. Using branched-DNA hybridization and the SCIP technique, we failed to observe HIV-1 enrichment at the nuclear periphery in a variety of cell types.

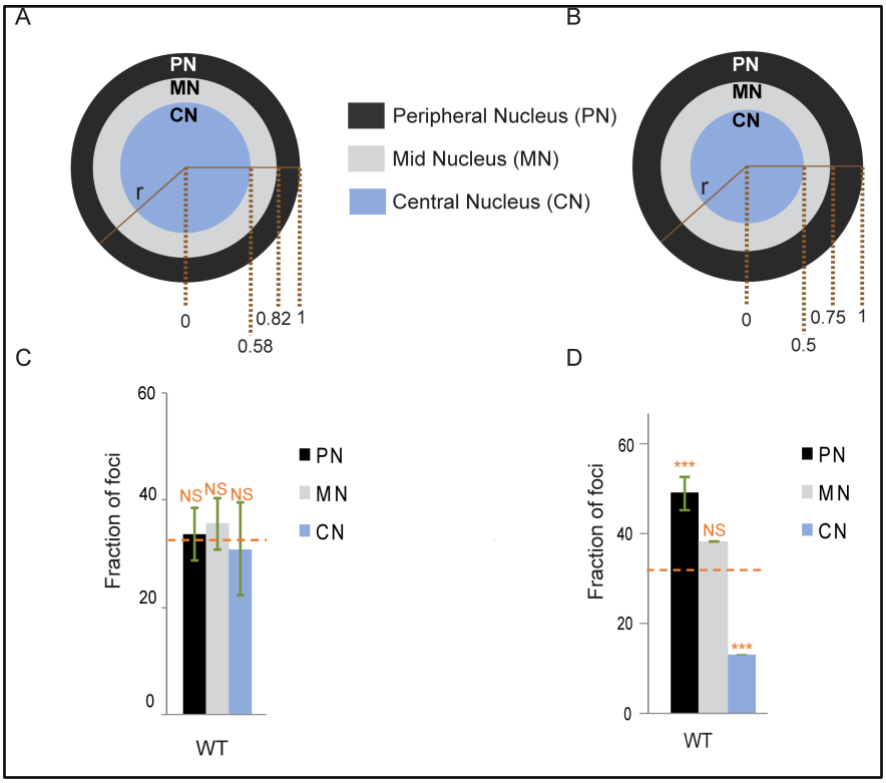

Fig.1 Meta-analysis with correct bin sizes downplays specific targeting of the PN during HIV-1 infection. (A) Nucleus is divided into three sections of equal area based on the measured radius (r). (B) The three sectional cutoffs used in [11] that marginally underrepresented the CN and inflated the true PN area. (C) and (D) Bar graphs displaying the proportion of viral DNA from a primary CD4+ T cell sample [11] binned into sectional areas as shown above in respective panels A and B. Orange dashed line indicates the expected random distribution among the PN, MN, and CN areas. *** $P < 0.0001$, ** $P < 0.01$ and NS $P > 0.05$ compared to random using Chi-Square test.

CA-CPSF6 interaction licenses HIV-1 to penetrate cell nuclei

Prior to our work, there was a disagreement regarding the potential role for LEDGF/p75 in the localization of HIV-1 within the nucleus, with two reports indicating that LEDGF/p75 played an important role in peripheral nuclear targeting [27, 30] and two other reports suggesting that LEDGF/p75 does not contribute to intranuclear localization [28, 29]. In terms of CPSF6, we had reported earlier that the interaction with CA was important for HIV-1 to penetrate into the nucleus [11]. To systematically analyze the roles of both cell proteins, we imaged viral DNA foci and mapped sites of HIV-1 integration using an isogenic set of HEK293T cells that were knocked out for LEDGF/p75, CPSF6, or both factors. We additionally visualized the intranuclear localization of preferred integration gene targets in uninfected HEK293T and primary CD4+ T cells. We did not observe a significant role for LEDGF/p75 in determining the localization of

HIV-1 inside the nucleus. However, loss of the interaction of CA with CPSF6 dramatically altered virus localization towards the nuclear periphery, with > 60% of HIV-1 foci locating to the PN area in CPSF6 knockout HEK293T cells and in CD4+ T cells infected with a CA mutant virus defective for the interaction with CPSF6. This shift in peripheral localization strongly correlated with integration of HIV-1 into transcriptionally inactive heterochromatin associated with the nuclear lamina [lamin-associated domains (LADs)] located at the nuclear periphery.

The role of CPSF6 in licensing HIV-1 PICs to transcriptionally active genes distal from the nuclear periphery became even more obvious when we analyzed the genes that are repeatedly targeted for HIV-1 integration under normal and CPSF6 depleted conditions. Under normal conditions, HIV-1 targeted comparatively small, transcriptionally active genes that dispersed throughout the nucleus. But when the CA-CPSF6 interaction was disrupted, this preference was lost. A unique set of genes that were larger, transcriptionally less active, and predominantly located in the PN area were enriched for HIV-1 integration upon CPSF6 depletion [22]. Furthermore, evidence for the role of CPSF6 in intranuclear targeting is evident from independent studies that imaged single HIV-1 particles inside the nucleus [3, 10, 11]. Francis and Melikyan [3] reported that while WT HIV-1 penetrated on average 1.8 µm from the NE in HeLa cells, mutant HIV-1 CA virus defective for the interaction with CPSF6 traveled on average only 0.5 µm from the NE. Using two-color STED microscopy, Bejarano et al. [10] reported that CPSF6 is recruited to HIV-1 PICs at the nuclear basket in primary CD4+ macrophages, which is essential for the PICs to release from the NE and travel inside the nucleus. They further observed that the majority of wild–type HIV-1 PICs upon CPSF6 depletion and HIV-1 CA mutant viral PICs defective for CPSF6 binding remained arrested at the NE, unable to enter the nucleus. Thus, the inability for PIC-associated CA to interact with CPSF6 renders HIV-1 unable to penetrate into cell nuclei, redirecting integration into chromatin in association with the nuclear lamina (Fig. 2). Roles for CPSF6 and LEDGF/p75 in the intranuclear localization of other lentiviruses, whether CPSF6 is initially recruited by HIV-1 in the cytoplasm or in the nucleus, and whether CPSF6 accompanies the PIC as it transits beyond the NE are active areas of investigation

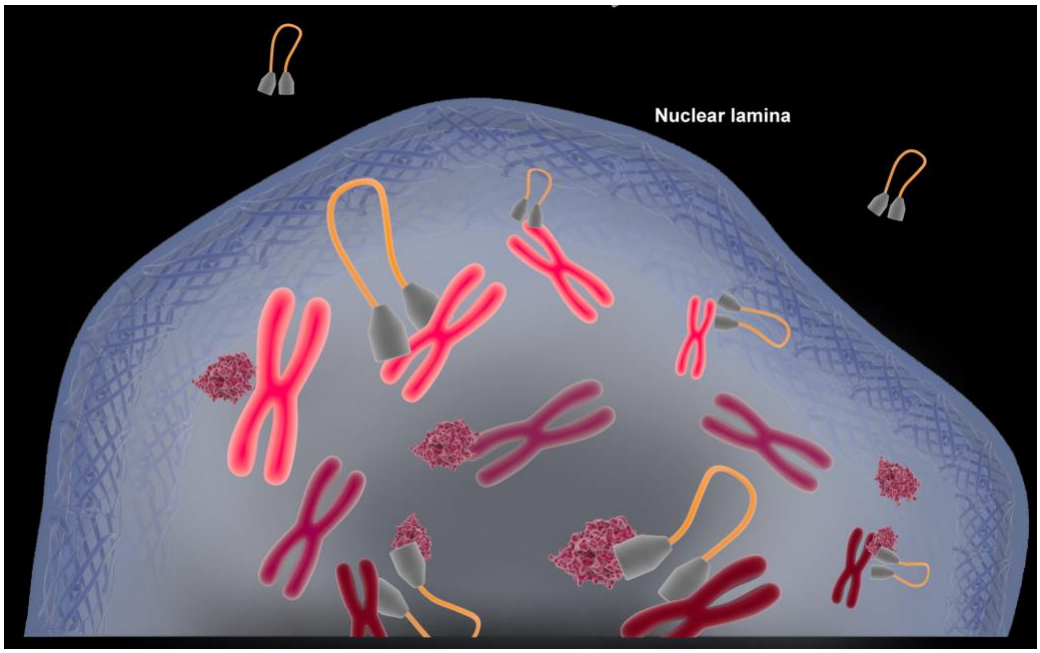

Fig.2 Inability to engage CPSF6 shifts HIV-1 localization and integration to the outer region of the nucleus. Engagement of CPSF6 (pink globules) by the HIV-1 capsid as part of the PIC (grey polygons with

orange DNA loop) is required to bypass the nuclear lamina (peripheral wire mesh) and access interior gene-dense regions of the genome (red chromosomes) for integration. Lack of CPSF6 engagement impedes penetration into the nucleus, resulting in integration into gene-sparse heterochromatin (shown as pink chromosomes) associated with the nuclear lamina.

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