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MEETING ABSTRACTS

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Oral presentations

Session 1: Entry and uncoating

O1

Visualization of the productive uncoating of single HIV-1 in living cells

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HIV-1 uncoating, which involves a partial or complete loss of capsid proteins (CA), is one of the most enigmatic steps in virus entry. By engineering a tetrameric CyclophilinA-DsRed (CypA-DsRed) fusion protein we generated a non-invasive label for the viral CA to report single HIV-1 uncoating [1]. Here, to identify productive uncoating events that lead to the virus nuclear import and infection, we performed extended time-lapse imaging and analysis of eGFP-encoding HIV-1 pseudoviruses co-labeled with INsfGFP and CypA-DsRed from 0 to 24 h post-infection. Single particle analysis of virus uncoating and nuclear import revealed that HIV-1 nuclear entry proceeds through steps of virus docking at the nuclear envelope (NE), followed by an accelerated loss of CypA-DsRed and nuclear penetration of INsfGFP complexes. The loss of CypA-DsRed at the NE reflected virus uncoating, since similar reduction in the CypA-DsRed fluorescence and in the CA signal of INsfGFP complex, as determined by immuno-fluorescence, is observed upon nuclear import. In agreement with the previous fixed cell studies, a subset of CypA-DsRed can remain associated with nuclear IN complexes and these complexes can be tracked for several hours, suggesting that HIV-1 undergoes terminal uncoating at the NE. Interestingly, however, a fraction of nuclear IN complexes disappears at varied times post-nuclear entry, and this loss of IN signal strongly correlates with subsequent expression of the eGFP reporter of infection. The N74D CA mutant, which uses alternative nuclear entry pathways, also uncoats at the NE, but fails to sufficiently penetrate into the nucleus and exhibits peripheral disappearance of IN complexes prior to eGFP expression. The > 3-fold slower kinetics of CypA-DsRed loss after the N74D mutant docking at the NE compared to wild-type viruses suggests the involvement of host factors at the NE in the accelerated uncoating and nuclear penetration of HIV-1. Collectively, our data demonstrate that CA-dependent steps of docking and uncoating at the NE are pre-requisites for HIV-1 nuclear import and infection. This work was supported by the NIH R01 grant AI129862 to G.B.M.

Keywords: Live cell microscopy; Capsid; Uncoating; Nuclear Import

Reference

1. Francis AC., Marin M., Shi J., Aiken C., Melikyan GB. Time-Resolved Imaging of Single HIV-1 Uncoating *In Vitro* and in Living Cells. PLoS Pathog. 2016; Jun 20; 12(6):e1005709.

O2

Mimicry of a +TIP binding motif by HIV-1 capsid coordinates early steps of infection

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Upon entry, HIV-1 exploits microtubule (MT) filaments for transport to the nucleus. Within the host cell, dynamic MTs continuously grow and shrink to explore the intracellular environment through a process of "search and capture". Their dynamic behavior is controlled by a small and highly specialized family of proteins known as plus-end tracking proteins (+TIPs). Although many viruses are known to exploit MTs for infection, how +TIPs might contribute to this process was unclear until recently. Our work provided the first direct evidence that a virus, HIV-1 actively stabilizes MTs by targeting two distinct +TIPs to control both its trafficking and uncoating. We found that soon after entry, the HIV-1 matrix protein binds the +TIP Kif4 to rapidly induce MT stabilization. This initial induction is further enhanced by incoming capsid (CA) targeting a second +TIP complex consisting of the formins, Diaphanous 1 and 2, offering a hand-off strategy for amplification of the levels of stable MTs as the virus proceeds through early infection.

Here, we tested the ability of other +TIPs such as cytoplasmic linker protein-170 (CLIP-170), known to promote MT growth as well as linkage to intracellular cargoes, and its partner dynactin (DCTN1) in influencing early HIV-1 infection. We found that while both CLIP-170 and DCTN1 bind in vitro assembled HIV-1 CA-NC complexes, these factors exert opposing effects on CA stability as well as early infection in multiple cell types including natural target cells, suggesting a potential competition between these factors for association with incoming capsids. Indeed, validating this competition, we found more CLIP-170 bound to CA-NC complexes in DCTN1 depleted cells while addition of DCTN1 reduced the amount of CLIP-170 on these complexes. In an attempt to understand why various +TIPs associate with HIV-1 capsid, domain analysis revealed the unexpected discovery of a common +TIP binding motif within HIV-1 capsid. Fusion of the housekeeping protein GAPDH to this +TIP binding homology sequence conferred on GAPDH the ability to interact with CLIP-170 or DCTN1. Collectively, our findings highlight how +TIP binding motif mimicry within HIV capsid creates functional modules for

many questions remain about its timing and location within the cellular environment.
A recently developed single virus imaging technique enables us to study labelled IN (IN-eGFP) inside individual viral complexes during the early stages of the replication cycle [1]. Different parameters including the number of fluorescent viral complexes, their distance to the nuclear envelope and intensity can be determined from these experiments. Although we can now identify single viral complexes in infected cells, we are unable to identify whether they contain reverse transcribed DNA. For this purpose, we combined our existing assay with the labelling of viral DNA using click chemistry. Ethynyl-functionalised nucleosides can be incorporated by RT and allow covalent linkage with azide reactive fluorophores via a copper-catalyzed azide-alkyne cycloaddition. In 2014, Peng and colleagues showed that it is possible to visualize HIV cDNA using 5-ethynyl-2′-deoxyuridine (EdU) [2]. However, this technique has some drawbacks. There is a high off-target labelling of the nucleus and cytoplasm due to EdU incorporation by the host DNA polymerases. Therefore, this technique is limited to the use in non-diving cells such as monocyte-derived macrophages (MDM) [3].
To this end, we opt to develop RT specific ethynyl-functionalised nucleosides that are not incorporated by the cellular DNA polymerases. With these analogues we could already lower the off-target labelling of the cellular DNA and reached up to 2% co-localisation of the total number of IN-eGFP with the viral DNA staining in HeLa P4. The RT specificity was already shown in an in vitro primer extension assay. Further validation of the analogue specificity will be done with a RT catalytic inactive mutant.
Keywords: HIV; DNA labeling; Click chemistry; Imaging

References

1. Borrenberghs, D. et al. Sci. Rep. 6, 1–14 (2016).
2. Peng, K., Muranyi, W., Glass, B., Laketa, V. & Yant, S. R. Elife 3, e04114 (2014).
3. Stultz, R. D., Cenker, J. J. & Mcdonald, D. J. Virol. 91, e00034-17 (2017).

P10

Flexible but conserved: a new essential motif in the CTD of HIV-1 group M integrases

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Several independent zoonotic transmissions have generated the different phylogenetic groups of HIV-1. Not all the groups have however undergone the same epidemiological success. Namely, HIV-1 group M is the one responsible for the pandemic, and group O is the second most abundant HIV, although with a largely lower epidemiologic success. The reasons for this discrepancy are only partially known to date.
HIV integrase (IN) catalyses the integration of the reverse transcribed viral DNA into the cell genome and has a central role in the control of the integration sites influencing the balance between latency of infection and viral replication. Being also involved in other steps of the infectious cycle, as reverse transcription, maturation of the viral particle and incorporation of the genomic RNA inside the viral core, the IN constitutes a keystone to the success of viral adaptation to new hosts.
Exploiting the natural genetic diversity existing among primary isolates of HIV-1 groups M and O, we have generated chimeras between IN of isolates of these groups and characterized their functionality. We have thus identified, in the C terminal domain of the enzyme, a new functional motif constituted of two lysines and two asparagines (NKNK motif). The NKNK motif is specific of group M isolates and is essential for integration. Indeed, the absence of lysines or asparagines results in decreased reverse transcription, marked reduction of nuclear import of reverse transcription products and reduced catalytic activity. A remarkable feature of the NKNK motif is its biochemical flexibility. Indeed, despite its strict conservation in vivo, the positions of the residues can be swapped in cell culture, often not affecting the integration process *per se*. This observation suggests that the motif could provide a surface of interaction with a partner yet to identify. Interestingly, though, the motif was not flexible with respect to reverse transcription, which was optimal exclusively with the canonical NKNK motif.
The biochemical versatility of this region of the integrase to carry out integration could have provided a major asset during viral evolution for acquiring additional functions during the infectious cycle, as its implication in reverse transcription.
Keywords: Integrase; Phylogenetic groups; Reverse transcription; Nuclear import

P11

The HIV-1 integrase interaction with Ku70 in the postintegrational gap repair

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The integration of the lentiviral DNA into the host genome is completed by cellular factors that repair the short gaps flanking the proviral DNA. Several repair complexes have been implicated to participate in the repair of the integration intermediate including components of the non-homologous DNA repair pathway (NHEJ) [1]. NHEJ is generally initiated by the binding of Ku heterodimer to a double strand DNA break. However, no DSBs are formed during lentiviral integration. We have shown earlier, that HIV-1 integrase can directly interact with Ku70 repair factor, and this interaction is weakened by E212A/L213A substitutions in integrase [2]. Overexpression of an integrase-binding domain of Ku70 in cells reduces the transduction by a single round replication incompetent CMV-driven HIV-1 vector but has no influence on the vector bearing the E212A/L213A substitutions. This effect may be caused by an inhibition of the interaction between integrase and the endogenous Ku70 protein by an integrase-interacting Ku70 domain. Using CRISPR/Cas9 technology we have established a set of 293T derived sublines with a stable depletion of either Ku70, Ku80 or DNA-PKcs subunits of the DSB detection complex. The depletion of any subunit resulted in a decrease in HIV-1 single cycle replication which be caused by a compromised integration or by a reduction in the postintegrational gap repair. To choose between these two possibilities, we have established a qPCR-based approach for a quantitative measurement of postintegrational gap repair. We have shown that depletion of any of the DNA-PK components reduced gap repair efficiency in our system. The same effect has been detected in the presence of specific DNA-PKcs inhibitor Nu7441 on 293T cells as well as on the lymphoid Jurkat cells. Viral vector carrying E212A/L213A integrase substitutions demonstrates decreased gap repair and a reduced sensitivity to the DNA-PK components depletion while its integrational capacity remains at the wild type level. We speculate that integrase recruits DNA-PK complex to gaps and facilitates gap repair through a direct interaction with Ku70 subunit.
This work has been supported by an RSF grant 17-14-1107 (preparation of knocked down cell lines and qPCR-based approach) and by an RFBR grant 18-34-0039.
Keywords: HIV-1; Integrase; Postintegrational repair; Ku

References
1. Knyazhanskaya E.S., Shadrina O.A., Anisenko A.A., Gottikh M.B. *Molecular biology*. V. 50 (4) **2016**, 567–579.
2. Anisenko A., Knyazhanskaya E. et al.*Scientific reports*. V. 7(1) **2017**, 5649.

P12

tRNA-assisted maturation of HIV-1 reverse transcriptase

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HIV-1 reverse transcriptase (RT) is a heterodimer consisting of two subunits p66 and p51, where subunit p51 is a product of HIV-1 Protease-catalyzed cleavage of the RNase H domain located at the C-terminus of p66. The processing of the p66/p66 homodimer to the mature p66/p51 heterodimer is a critical step in virus maturation and reverse transcription, and is essential for enabling infectivity. The proteolytic cleavage site in p66 subunit is sequestered in the middle of a β-sheet and inaccessible to the protease in the known p66/p51 RT structures. The molecular mechanism of how the mature p66/p51 heterodimer is formed is unknown. Here we report that the proteolytic processing of the RT p66/p66 homodimer to the mature p66/p51 heterodimer is significantly facilitated by interaction of the homodimer with tRNA. Other RNAs molecules have considerably less pronounced effect. Based on our biochemical and structural data, we propose a model in which interaction of the p66/p66 homodimer with tRNA introduces conformational asymmetry in the two subunits, permitting specific proteolytic processing of one of the two p66 subunits in p66/p66 to form the p66/p51 heterodimer.
Keywords: HIV-1 reverse transcriptase, p66, maturation, tRNA

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P13

Studies on HIV Reverse Transcriptase inhibitory activity of isatin derivatives

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Background: HIV Reverse Transcriptase (HIV RT) plays important roles in HIV virus replication, including conversion of viral RNA into cDNA. Identification of novel inhibitors of HIV Reverse Transcriptase has emerged as potential antiviral agents for the treatment of HIV/AIDS. Present work is to investigation of HIV Reverse Transcriptase inhibitory activity of isatin derivatives (2,3-dioxoindole) and its fused compounds
Method: Isatins and its condensed compounds indeno and Indolo (fused isatin compounds) were investigated for inhibition of HIV Reverse Transcriptase enzymatic activity to understand the mechanism of antiviral action.
Results: All compounds exhibited inhibitory activity against HIV-1 Reverse Transcriptase (25–95% inhibition at 100 µg/mL). The 5-bromo isatin and 5-chloro isatin displayed significant inhibitory activity against HIV RT enzymatic activity (66 and 95 at 100 µg/mL), respectively, whereas standard Nevirapine was found to be 99% activity at 10 µg/mL. The fused isatin derivatives such as Indolo and Indeno derivatives less active than isatin derivatives.
Conclusion: All the isatin derivaties inhibit the HIV Reverse Transcriptase (HIV RT) activity and 5-chloroisatin had significant activity against HIV 1 Reverse Transcriptase and ring fusion in isatin molecules leads to loss the HIV RT activity
Keywords: HIV 1; HIV RT; Isatin

Reference
1. Selvam P, Murugesh N, Chandramohan M, Debyser Z, Witvrouw M.Design, Synthesis and antiHIV activity of Novel Isatine-Sulphonamides.*Indian J Pharm Sci.* **2008** Nov;70(6):779–82

Table 1. *In vitro* Anti-HIV-RT activity

Sample ID	% Inhibition at 10 µg/ml	% Inhibition at 100 µg/ml
Isatin	16.49	46.36
5-Chloro Isatin	39.10	95.09
5-Bromo Isatin	30.42	66.09
5-Chloro Indolo	17.83	25.90
Indeno	22.07	41.22
Nevirapine	99.61	

P14

Investigation of anti-HIV RT activity and anti-HIV activity of Poly Herbal Powder BH

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Background: HIV Reverse Transcriptase (HIV RT) plays important roles in several steps of HIV virus replication, including conversion of viral RNA into cDNA. Identification of novel inhibitors of HIV Reverse Transcriptase has emerged as promising antiviral agents for the treatment of HIV/AIDS. Present work is to investigation of anti-HIV activity and HIV Reverse Transcriptase inhibitory activity of various extracts of polyherbal powder (BH).
Method: Ethanolic extract of Polyherbal powder (BH-ET) were tested for anti-HIV activity against HIV-1(NL 4.3) replication in 5000 TZM B1 cells. BH extracts were investigated for inhibition of HIV Reverse Transcriptase enzymatic activity to understand the mechanism of antiviral action.
Results: All extracts exhibited inhibitory activity against HIV-1 Reverse Transcriptase (28–71% inhibition at 100 µg/mL). The ethanolic extract (BH-HT) and Hexane extract (BH-H) displayed significant inhibitory activity against HIV RT in enzymatic activity (63 and 71 at 100 µg/mL), respectively, whereas standard Nevirapine was found to be 99& at 100 µg/mL. The ethanolic extract also had 60% inhibition of the HIV-1 replication at the concentration of 100 µg/mL and standard AZT had 100% inhibition at 5 µM.
Conclusion: All the extracts inhibit the HIV Reverse Transcriptase (HIV RT) activity and ethanolic extract (BH ET) inhibit both HIV 1 replication and Reverse Transcriptase.
Keywords: HIV 1; HIV RT; Poly herbal Powder (BH); TEM B1 cells

Reference
1. Balraj S., Selvam P., Pommier Y., Metifiot M., Christophe M., Pannecouque C., and De Clercq E. Investigation of anti-HIV activity, cytotoxicity and HIV integrase inhibitory activity of polyherbal formulation BH extracts. *BMC Infect Dis.* **2014**; 14(Suppl 3): O23