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# Spatial juxtaposition of HIV-1 provirus with PML and KAKA bodies as revealed by 3D Immuno DNA FISH

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Various members of tripartite motif (TRIM) protein family display antiviral properties, targeting retroviruses in particular. The potential activity of TRIM19, better known as promyelocytic leukemia protein (PML) against several viruses has been well documented, yet it's role in HIV-1 infection remains elusive.

One of the most important cellular partners of HIV-1, P-TEFb kinase complex, composed of CDK9 and CyclinT1 plays a crucial role in regulation of HIV-1 transcription. We have previously demonstrated that both members of P-TEFb interact with PML protein and localize inside the PML Nuclear Bodies (PML NB) [1,2]. In particular, we found that the acetylated and enzymaticaly inactive form of CDK9 binds PML and can be separated with PML in the insoluble chromatin fraction. Our ChIP analysis revealed that acetylated form of CDK9 localizes to the integrated and transcriptionally silent viral genome, thus indicating that PML might have yet unidentified role in regulation of HIV-1 infection and latency [2].

We indeed identified PML protein bound to the silent viral genome in Jurkat cells harboring single integrations of HIV-1 provirus. The distribution of PML protein along the viral genome could be correlated with bimethylated lysine 9 (K9) on Histone 3 (2MetK9 H3), a mark of facultative heterochromatin. Transcriptional activation of the provirus with TNF-α led to a displacement of PML protein from the genome and to a substantial loss of 2MetK9H3 mark. By three-dimensional immuno fluorescent in situ hybridization (3D Immuno DNA FISH) that we developed we visualized the viral genome in close proximity to PML NB and we characterized this position as adjacent. Within the population of cells tested in the silenced state, 40% of the cells showed proximity at the distance of less that 0.6μm. PML NB were recently described to have a close spatial relationship with the so called KAKA foci, characterized by the presence of KAP-1 (or Trim28) and KRAB-Zinc Finger proteins [3] thus prompting us to analyse the relationship of the silent HIV-1 provirus with these nuclear structures. We detected numerous PML and KAKA foci in both Jurkat cells (J-LAT model of latency) and primary human $CD4^+$T lymphocytes, with HIV-1 residing either in close proximity to PML or to KAKA foci (~40% showing less than 0.6μm distance) in J-LAT latency model. Whether this spatial localization of HIV-1 provirus is correlated to silencing of the virus genome or is a general feature of HIV-1 integration and replication remains still to be determined.

## References

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