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ROLE OF PML NUCLEAR BODIES DURING MOUSE POLYOMAVIRUS INFECTION: PML PROTEIN ISOFORMS AND THE NON-CANONICAL HISTONE H3.3

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The role of promyelotic nuclear bodies (PML NBs) in virus infection have been intensively studied. However, their possible restriction function and the role of proteins associated with PML NBs is not well understood yet. The first aim of this study was to characterize the possible restriction function of the main structural component of PML NBs, PML protein, and its individual isoforms during Murine polyomavirus (MPyV) infection. The mouse *PML* gene is composed of nine exons that are alternatively spliced to produce isoforms. Only 3 isoforms (mPML1-3) have been described in the mouse model. At early times post MPyV infection, we observed mPML NBs to localize in close proximity to MPyV transcriptional and replication centres. In *PML* KO cells, transcription from the early gene promoter was more efficient in comparison with that in wt cells, suggesting trascription restriction by PML protein(s). During transient expression, the largest isoform, mPML2, became incorporated into endogenous PML NBs and also in *Pml* KO cells, it formed speckles. Nevertheless, the overexpression of mPML2 isoform did not significantly affect MPyV infection in both *Pml* KO and wt cells. Further, we detected cellular expression of two predicted mPML isoforms, mPMLX4 and mPMLX6, and one novel isoform, named by us mPMLXK. Further studies have been carried out to evaluate their functions. The second aim of this study was to reveal the possible participation of proteins transiently interacting with PML NBs, chaperones HIRA and DAXX/ATRX, in deposition of non-cannonical histone H3.3 into viral minichromosomes and the functional consequences of such incorporation. We found H3.3 incorporated not only in condensed minichromosomes in virions but also accumulated at the sites of MPyV replication. It suggests that incorporation of H3.3 to the viral chromatin is important either for the regulation of viral expression or for genome packaging. Further, the massive recruitment of DAXX to the sites of MPyV replication was observed. Hovewer, the absence of PML or DAXX did not prevent the H3.3 incorporation into viral minichromosomes. This indicates the contribution of other chaperones, e.g. HIRA, to the deposition of H3.3 into the viral minichromosomes.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.