ID de Contribution: **3** Type: **Non spécifié**

DNA minicircles are new tools to study in vitro retroviral integration

Integration is an essential step of retroviral replication and a promising anti-HIV target. It is performed by a viral-encoded enzyme, integrase (IN), and the selectivity of this enzyme for the target cellular DNA is important for retroviral replication. Genetic, biochemical and structural studies have revealed a role of cellular chromatin in this selectivity but the underlying molecular parameters are still under investigation. Indeed, at the level of the nucleosome, both histone modifications specifically recognized by IN and its cofactor LEDGF/p75 and DNA distortions induced by the nucleosome structure are involved in the distribution of integration sites. To distinguish between these two parameters, we constructed DNA minicircles that reproduce the DNA curvature and torsion present in a nucleosome, but lack the histones. Using various DNA minicircles as integration susbtrates, we observed a large enhancement of integration in these circles with regards of the corresponding linear fragments. This enhancement is observed with both HIV-1 IN and PFV IN but also with the HIV IN-LEDGF/p75 complex. Using high-throughput sequencing of integration products obtained in the DNA minicircles and molecular modelling of these circles, we evaluated the role of DNA structural parameters on the selectivity of integration. With the HIV IN-LEDGF/p75 complex, we observed a periodic distribution of integration sites enriched in outward DNA major grooves, which is consistent with the cryo-EM structure of the intasome obtained with this complex. Surprisingly, this periodicity is less pronounced with HIV IN alone and absent with PFV IN. Using the DNA minicircles, we are now studying the role of the LEDGF/p75 cofactor and IN residues suspected to be involved in the recognition of curved or flexible acceptor DNA substrates. Using under and over-twisted DNA minicircles, we will also evaluate the effect of local DNA superhelicity on integration.
In summary, DNA minicircles allow to study how local structural deformations of the target DNA affect retroviral integration and to determine the separated roles of DNA structure and histone modifications during this enzymatic process.

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