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SELECTIVE INHIBITION OF MOLONEY MURINE LEUKEMIA VIRUS CYCLE AT THE INTEGRATION STEP BY THE NETROPSINE-INTERCALATING HYBRID MOLECULE NET-OPC

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In view of the development of new antiviral agents acting through the artificial modulation of some genomic functions, two hybrids molecules composed of a *bis*-pyrrolecarboxamide chain related to netropsin, linked to the intercalating chromophore oxazolopyridocarbazole have been synthesized (Mrani et al. 1991, Subra et al. 1991) and further tested on the cycle of a defective Moloney Leukemia Virus (Psi-Neo; M.MuLV) derivated from the SVX shuttle (Cepko et al. 1985) and expressing when integrated in the host DNA cellular genome the resistance to the G418 antibiotic. The drug (Net-OPC) displaying a binding preference to duplex DNA containing A+T bases and recognizing 3'CTT containing regions, markedly inhibits the retroviral replicative cycle. In contrast the related molecule GM283 devoided of sequence preference displays no significant action on the viral cycle. Using the *neo* gene amplification by quantitative PCR as probe, it is demonstrated that Net-OPC selectively inhibits the process leading to the integration of the viral DNA into the host 3T3 genome. This effect is suggested to come from the binding of the drug to the viral DNA CTTTC region of the LTRs which is a part of the presumed binding site of the IN protein which catalyze the integration of the viral DNA in the genomic cellular DNA (Colliceli & Goff, 1985; Basu & Varmus, 1990,; Bushmann & Craigie, 1990).

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Characterization of Phosphorylation of HIV Reverse Transcriptase
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Phosphorylation of proteins has been shown to modulate the activities of many important enzymes, among these are eukaryotic DNA and RNA polymerases. We have investigated the phosphorylation of HIV reverse transcriptase (RT) *in vitro* and in an *in vivo* model, the Xenopus oocytes. *In vitro*, HIV RT was shown to be a substrate for cAMP-dependent protein kinase (PKA). RT phosphorylated by PKA was about 10-fold more active than unphosphorylated RT in the presence of 100 mM KCl. The K_m of phosphorylated RT for dTTP was 3-fold lower than the unphosphorylated RT. The stoichiometry of the reaction was shown to be one phosphate to one RT monomer. Both subunits of RT were phosphorylated to the same extent. The *in vivo* model employed Xenopus oocytes incubated in medium containing ^{32}P. Purified RT was phosphorylated when it was microinjected into the oocytes. Peptide maps prepared from RT phosphorylated *in vivo* and *in vitro* by PKA were similar, suggesting that PKA might be the enzyme responsible for *in vivo* phosphorylation in oocytes. Peptide fragments were made from HIV RT phosphorylated by PKA using V-8 protease and chymotrypsin. The ^{32}P labeled fragments were purified by HPLC for sequencing analysis and mass-spectrometry. These analyses suggested that Ser 105, one of the three PKA consensus sites in RT, was the site of phosphorylation . This conclusion was confirmed by site specific mutagenesis altering Ser to Ala at amino acid 105 which resulted in no phosphorylation. Thus,cellular mediated phosphorylation of RT may play an important role in the reverse transcription of the HIV genome.