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Analysis of LEDGF/p75 expression regulation

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Background

To replicate, retroviruses must insert DNA copies of their RNA genomes into the host genome. This integration process is catalyzed by the viral integrase protein. The site of viral integration has been shown to be non-random and retrovirus-specific. LEDGF/p75, a splice variant encoded by *PSIP1* gene and described as a general transcription coactivator, was identified as a tethering factor binding both to chromatin and to lentiviral integrases, thereby affecting integration efficiency as well as integration site selection. LEDGF/p75 is still a poorly characterized protein, and its cellular endogenous function has yet to be fully determined. In order to start unveiling the roles of LEDGF/p75 in the cell, we started to investigate the mechanisms involved in the regulation of LEDGF/p75.

Materials and methods

To identify *PSIP1* minimal promoter and associated regulatory elements, we cloned a region starting 5 kb upstream the transcription start site (TSS, +1 reference position) to the ATG start codon (+816), as well as systematic truncations, in a plasmid containing the *firefly luciferase* reporter gene. These constructs were co-transfected into HEK293 cells with a plasmid encoding the *Renilla luciferase* under the pTK promoter as an internal control for transfection efficiency. Both luciferase activities were assessed by luminescence as an indicator of promoter activity.

Results

Luciferase assays identified regions -76 to +1 and +1 to +94 as two independent minimal promoters showing respectively a 3.7x and 2.3x increase in luciferase activity. These two independent minimal promoters worked synergistically increasing luciferase activity up to 16.3x as compared to background. Moreover, we identified five regulatory blocks which modulated luciferase activity depending on the DNA region tested, three enhancers (-2007 to -1159, -284 to -171 and +94 to +644) and two silencers (-171 to -76 and +796 to +816). However, the silencing effect of the region -171 to -76 is dependent on the presence of the +94 to +644 region, ruling out the enhancer activity of the latter. Computational analysis of *PSIP1* promoter revealed the absence of TATA box and initiator (INR) sequences, classifying this promoter as non-conventional. TATA-less and INR-less promoters are characterized by multiple Sp1 binding sites, involved in the recruitment of the RNA pol II complex. Consistent with this, *PSIP1* promoter contains multiple putative Sp1 binding sequences in regions -76 to +1 and +1 to +94.

Conclusion

Based on these data, we proposed a model for *PSIP1* promoter activity involving a complex interplay between yet undefined regulatory elements to modulate gene expression.