RETROTRANSPOSON Tf1 IS TARGETED TO Pol II PROMOTERS BY TRANSCRIPTION ACTIVATORS

Young Eun Leem, Felice Kelly, Hirotaka Ebina, Tracy Ripmaster, Marc Heincelman, Ke Zhang[1], Shiv Grewal[1], Charles Hoffman[2], and Henry Levin

Lab of Gene Regulation and Development, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892; [1]Lab of Biochemistry and Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20892; [2]Department of Biology, Boston College, Chestnut Hill, MA 02467

Because of their similarities to retroviruses, long terminal repeat (LTR)-retrotransposons are important models for retrovirus replication. The retrotransposon under study in our laboratory is the Tf1 element of the fission yeast *Schizosaccharomyces pombe*. We are particularly interested in Tf1 because of its strong preference for integrating into pol II promoters. This choice of target sites is similar to the strong preferences HIV-1 and MLV have for integration into pol II transcription units. To determine which features at insertion sites are recognized by the transposon we developed a plasmid-based method for measuring integration into specific sequences. We tested five different genes with this in vivo assay and found Tf1 was targeted specifically to sites upstream of each ORF. Target assays of the *fbp1* gene showed the insertions occurred adjacent to the binding site for the Atf1p/Pcr1p complex of transcription activators. Mutations in the binding site for Atf1p/Pcr1p as well as a deletion of the *atf1* gene resulted in substantial reductions in integration at *fbp1*. These suggest integration is targeted to the promoter of *fbp1* by a tethering activity of Atf1p. Additional support for this idea came from studies of recombinant Atf1p and integrase that revealed the central core of integrase bound the basic leucine zipper (b-ZIP) of Atf1p. The results of gel mobility assays demonstrated that Tf1 integrase forms a complex with Atf1p and DNA from the *fbp1* promoter. This complex was disrupted by mutations in the DNA at the Atf1p recognition site. To determine which promoters throughout the genome of *S. pombe* are targets of Tf1 integration we used ligation mediated PCR combined with ultra high throughput sequencing. The 100,000 integration sites identified in the genome of *S. pombe* exhibited the same strong preference for promoter regions as observed previously. The positions of insertion were broadly distributed throughout the chromosomes and in one experiment 50% (2505) of all intergenic sequences contained insertions. The number of insertions in each promoter varied widely. Four independent integration experiments demonstrated that the levels of integration activity of each intergenic regions were highly reproducible. Our analysis of these data indicates that each promoter possesses an intrinsic level of target activity and this activity does not correlate with transcription activity.