EVALUATION OF INTEGRASES IN THE CONTROL OF GENE REGULATION IN MAMMALIAN CELLS: A TOOL FOR THE CONSTRUCTION OF SYNTHETIC BIOLOGICAL CIRCUITS

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ABSTRACT:

Serine-integrases were identified as responsible for the unidirectional integration of the bacteriophage genome into the bacterial DNA. This insertion occurs between specific sites designated as attB (bacterium) and attP (phage), which after the interaction are merged and form the new attL/attR sites. Aiming at its use as a biotechnological tool for gene regulation, those sites can be positioned flanking a gene of interest. Once the attB/attP sites are recognized, the integrase reverses this sequence, placing it in a reverse complementary position allowing its expression. Previous studies have demonstrated effective to turn on/off a reporter gene in prokaryotic cells using different integrases. However, knowledge about function of integrases in eukaryotic cells are still limited. So, this work aimed to evaluate the functionality of integrases in the control of gene expression in mammalian cells for the construction of genetic circuit. HEK 293T cell line and bovine skin fibroblast cells were transfected with six integrases in a plasmid co-transfection system, as follows: one vector containing the integrase and the second vector containing the reporter gene (GFP) in inverted orientation, flanked by attB/attP sites. After transfection, cells were incubated for 48 h, and GFP expression was evaluated by fluorescence microscopy and flow cytometry. The CDS inversion was analyzed by PCR followed by DNA sequencing. The results showed the functionality of three integrases. These results may have potential for the modulation of gene expression and to expand the possibilities of design and application of synthetic genetic circuits in mammalian cells.

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