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Effect of mutations in a simian virus 40 PolyA signal enhancer on green fluorescent protein reporter gene expression

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ABSTRACT. Our previous studies have shown that tandem *Alu* repeats inhibit green fluorescent protein (GFP) gene expression when inserted downstream of the GFP gene in the pEGFP-C1 vector. We found that the 22R sequence (5'-GTGAAAAAAATGCTTTATTTGT-3') from the antisense PolyA (240 bp polyadenylation signal) of simian virus 40, eliminated repression of GFP gene expression when inserted between the GFP gene and the *Alu* repeats. The 22R sequence contains an imperfect palindrome; based on RNA structure software prediction, it forms an unstable stem-loop structure, including a loop, a first stem, a bulge, and a second stem. Analysis of mutations of the loop length of the 22R sequence showed that the three-nucleotide loop (wild-type, 22R) induced much stronger GFP expression than did other loop lengths. Two mutations, 4TMI (A7→T, A17→T) and 5AMI (A6→T, T18→A),

which caused the base type changes in the bulge and in the second stem in the 22R sequence, induced stronger GFP gene expression than 22R itself. Mutation of the bulge base (A17→T), leading to complete complementation of the stem, caused weaker GFP gene expression. Sequences without a palindrome (7pieA, 5'-GTGAAAAAAATG CAAAAAAAGT-3', 7pieT, 5'-GTGTTTTTTTTGCTTTTTTTGT-3') did not activate GFP gene expression. We conclude that an imperfect palindrome affects and can increase GFP gene expression.

Key words: Stem-loop; GFP; Alu; SV40 PolyA; Mutation

