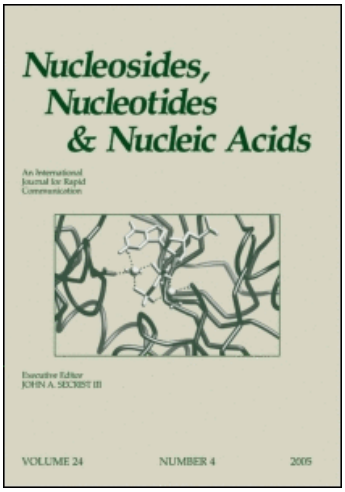

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EFFECTIVE SUPPRESSION OF HIV-1 GENE EXPRESSION BY A MAMMALIAN tRNA 3′ PROCESSING ENDORIBONUCLEASE AND EXTERNAL GUIDE SEQUENCE OLIGOZYMES

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ABSTRACT

We examined the suppression of virus expression by cleaveage of the HIV-1 RNA gene using a mammalian tRNA 3′ processing endoribonuclease and an External Guide Sequence Oligozyme (EGS) *in vivo*. We constructed an EGS expression vector that used the $tRNA^{met}$ promoter as an expression cassette for EGS. The EGS expression vector was targeted to the upstream region of gag, region. The EGS expression vector was co-transfected into COS cells with the HIV-1 gene plasmid vector. As compared with the EGS non-expressing cells and the EGS expressing cells, the EGS expressing cells with the targeted gag start codon had a clearly decreased amount of the HIV-1 gag p24 protein. The EGS expressing cells with the targeted gag start codon showed effective suppression of HIV-1 gene expression. Thus, these studies describe novel gene targeting agents for the inhibition of gene expression and antiviral activity.

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INTRODUCTION

Antisense technology is a promising targeting approach for use in basic research and clinical therapeutic applications. The gene targeting agents can be a conventional antisense oligonucleotide, an antisense catalytic molecule (ribozyme or DNA enzyme), or an antisense molecule with an additional (guide) sequence that targets the mRNA for degradation by endogenous RNases (1,2).

Mammalian tRNA 3′ procressing enzymes (3′ tRNase) have been reported. These enzymes can remove a 3′ trailer after the discriminator nucleotide from various precursor tRNAs (3). It has been reported that the enzyme could be converted to a highly specific endoribonuclease using tRNAs can that have been artificially 3′ truncated. Indeed, the 3′ tRNase can cleave a 3′ half tRNA (consisting of the 3′ trailer) in the presence of a 5′ half tRNA (EGS, consisting of the 5′ end) (4).

Using a two half-tRNA system, we demonstrated that the 3′ tRNase could recognize and cleave pre-tRNA containing various HIV-1 RNA targets *in vivo* (Fig. 1). In this study, we describe the intracellular inhibition of HIV-1 expression using EGS.

RESULTS AND DISCUSSION

At first, the EGS was inserted into pSV2neo, which contained the sequence of the $tRNA^{met}$ promoter region. The EGS base sequence was confirmed using the ABI PRISM™ 310 Genetic Analyzer (PE Biosystems). The EGS expression vectors were transfected into COS cells with the HIV-1 genome, and the suppression of HIV-1 gene expression was examined. Figure 2 shows the result, regarding the expression rate of HIV-1 p24. As compared with the EGS non-expressing vector and the EGS expressing cells, the EGS expressing cell with the targeted gag start codon had a clearly decreased amount of the p24 protein. This region showed more effective suppression of HIV-1 gene expression than the EGS expression vector with the targeted gag p24 region. We thought that the EGS-gag p24 was not

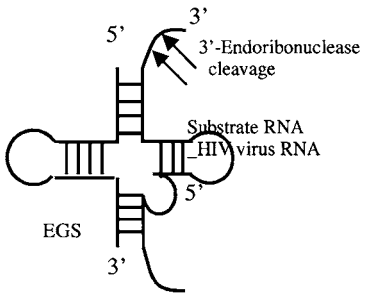

Figure 1. Specific cleavage of target RNAs with EGS by mammalian tRNA 3′ processing endoribonuclease.

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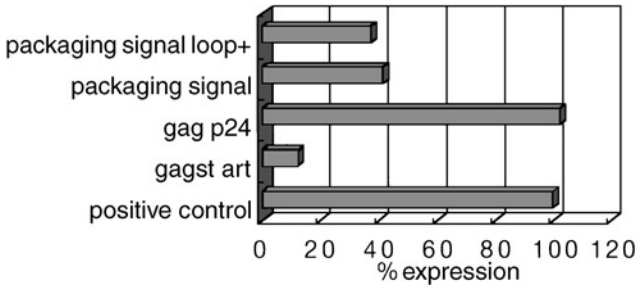

Figure 2. Rate of HIV-1 p24 expression.

complementary to the target RNA, and did not form a pre-tRNA-like complex. For this reason, EGS-gag p24 showed no 3′-endoribonuclease activity. Therefore, the EGS expression vector with the targeted gag start codon region is useful as a gene inactivating agent of HIV-1.

EXPERIMENTAL SECTION

The EGS expression vector that uses the $tRNA^{met}$ promoter was constructed as follows. The EGS was designed to be targeted to the gag region of HIV-1 RNA. EGS antisense and sense oligonucleotides that encoded the terminator were annealed and ligated into the *Kpn*_/*Bam* H_ sites of pSV2neo, which contained the sequence of the $tRNA^{met}$ promoter region. These plasmids were isolated from *E.coli* XL2-blue and were purified on a JETSTAR Plasmid Purification System (GENOMED Inc.). These EGS base sequences were analyzed using an ABI PRISM^{TM} 310 Genetic Analyzer (PE Biosystems).

COS cells were grown in RPMI 1640 (SIGMA) supplemented with 10% heat–inactivated fetal bovine serum (FBS) at 37_ in a 5% CO_2 atmosphere. Plasmids expressing the EGS, the luciferase gene, and other parts of the HIV-1 genome (except *env* and *nef*) were co-transfected into COS cells. The COS cells (20_10^4 cells/2 ml) were prepared and transfected with the EGS expression vector using the FuGENE^{TM} 6 transfection reagent (Roche Diagnostics K.K.). After 48 hours, the COS cells were harvested and the luciferase activities and the amounts of HIV-1 gag p24 in these cells were measured.

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REFERENCES

1. Plehn-Dujowich, D.; Altman, S. *Proc Natl Acad Sci USA*., **1998, 95**, 7327–7332.
2. Yuan, Y.; Altman, S. *Science*, **1994, 263**, 1269–1273.
3. Nashimoto, M. *Nucleic Acids Res*., **1997, 25**, 1148–1154.
4. Nashimoto, M. *RNA*, **1996, 2**, 523–534.

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