Development and application of siRNA expression vector

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ABSTRACT
RNA interference (RNAi) is a sequence-specific silencing phenomenon, which is induced by double-stranded RNA (dsRNA) and mediated through an evolutionarily conserved mechanism from plants to mammals. In mammalian cells, it has recently been reported that 21- or 22-nucleotide (nt) RNAs with 2-nt 3' overhangs (siRNA) induce RNAi without induction of the dsRNA-dependent inhibition of protein synthesis, known as the host defense system against viral infections. Moreover, we and other have developed siRNA expression systems utilizing a pol III promoter. Here we report a comparative analysis among various siRNA expression vectors and also demonstrate a regulatable RNAi in cells by using a tetracycline-controlled U6 promoter.

INTRODUCTION
Gene silencing by dsRNA was first discovered in Caenorhabditis elegans with careful and elegant analysis (1). This phenomenon, called RNAi, was also observed subsequently in various organisms including plants, Drosophila and a protozoan. Although the mechanism of RNAi is not fully understood, recent genetic and biochemical studies have unveiled the mechanism at the molecular level. In cells, dsRNAs are digested into fragments of 21-23 nucleotides (nt) with 2-nt 3' overhangs by an RNase III-related enzyme, which has been recently identified as Dicer in Drosophila (2). Subsequently, the resulting small interfering RNAs (siRNAs) are incorporated into protein/RNA complexes (3), or RISCs (RNA-induced silencing complexes) (4), and appear to be unwound to single-stranded RNAs (3) that act as guide sequences. The active complexes containing the guide RNA recognize and cleave the target RNA.

RNAi was used initially as a tool to define the functions of unknown genes in various organisms, but the silencing of specific genes by RNAi was not easily detectable in mammalian systems. Introduction of dsRNA into mammalian cells triggers the dsRNA-dependent nonspecific inhibition of protein synthesis, namely the host defense system against viral infections. However, Tuschl and colleagues made important progress towards overcoming this problem, and soon it was demonstrated that 21- or 22-nt RNAs with 2-nt 3' overhangs, called siRNAs, induce gene silencing without inducing the nonspecific inhibition of translation in cultured mammalian cells (5, 6). More recently, we and others have developed vector systems capable of producing siRNAs for inducing RNAi in mammalian cells (7, 8). These vector systems utilize the Pol III promoter, the small nuclear RNA U6, or the human RNase P RNA H1 promoter and were classified into two types, tandem-type and stem-type, corresponding to the form of the transcribed small RNA molecule(s) (Figure 1). In this report, we compare the various siRNA expression vector systems using a dual luciferase assay, and we also demonstrate a controlled RNAi by using a tetracycline-controlled U6 promoter.

RESULTS AND DISCUSSION
To compare various types of siRNA expression systems, we constructed tandem-type or stem-type siRNA expression vectors under the control of the H1 or U6 promoter, which were targeted against the same site with the firefly luciferase gene. HeLa cells were cotransfected with a Renilla luciferase plasmid, a firefly luciferase plasmid, and each siRNA expression vector. The results of the luciferase assay showed that all four constructs efficiently suppressed firefly luciferase gene expression (80% to 90%) at high dose of siRNA expression plasmid. The
suppressive activities of U6-directed siRNA vectors showed slightly higher levels than those of H1-promoter siRNA vectors in each case of both stem-type and tandem-type. When used at low dose of siRNA expression plasmid, it was observed that the stem-type siRNA expression vector was more potent than the tandem-type siRNA expression vector. This may reflect the difference of the hybridization efficiency between sense and antisense RNAs produced by the stem-type and tandem-type.

We next tried to regulate RNAi by using the tetracycline-regulated U6 promoter, which was previously characterized in our laboratory. We constructed a firefly luciferase-targeted siRNA expression vector under the control of the tetracycline-regulated U6 promoter. HEK293/TetR cells, which stably express the gene for the tetracycline repressor, were cotransfected with the tetracycline-controlled siRNA expression vector, the firefly luciferase expression vector, and the Renilla luciferase expression vector in the presence or absence of tetracycline. The suppressive activity of wild-type siRNA expression vector was unaffected by the presence or absence of tetracycline. In contrast, the construct with the tetracycline-regulated U6 promoter clearly inhibited the expression of firefly luciferase in the absence of tetracycline, but did not suppress gene expression in the presence of tetracycline (Figure 2). This result demonstrates that the siRNA expression system with the tetracycline-regulated U6 promoter(s) can indeed have potential to control RNAi in cells.

Figure 1. Schematic representation of siRNA expression vectors. (A) Each U6 or H1 promoter directs the transcription of sense and antisense RNAs, which are subsequently annealed and form siRNA duplexes. (B) H1 or U6 promoter directs the transcription of hairpin RNAs, which are processed by ribonuclease(s) and form siRNA duplexes.

Figure 2. Control of RNAi by the tetracycline-regulated U6 promoter. (A) Schematic diagram of constructs used in the experiment. (B) HeLa S3 cells were cotransfected with firefly luciferase expression vector, Renilla luciferase expression vectors and pU6i or pU6Teti in the presence or absence of tetracycline.

REFERENCES