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A novel bidirectional expression system for simultaneous expression of both the protein-coding genes and short hairpin RNAs in mammalian cells

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Abstract

RNA interference (RNAi) is an extremely powerful and widely used gene silencing approach for reverse functional genomics and molecular therapeutics. In mammals, the conserved poly(ADP-ribose) polymerase 2 (PARP-2)/RNase P bidirectional control promoter simultaneously expresses both the PARP-2 protein and RNase P RNA by RNA polymerase II- and III-dependent mechanisms, respectively. To explore this unique bidirectional control system in RNAi-mediated gene silencing strategy, we have constructed two novel bidirectional expression vectors, pbiHsH1 and pbiMmH1, which contained the PARP-2/RNase P bidirectional control promoters from human and mouse, for simultaneous expression of both the protein-coding genes and short hairpin RNAs. Analyses of the dual transcriptional activities indicated that these two bidirectional expression vectors could not only express enhanced green fluorescent protein as a functional reporter but also simultaneously transcribe shLuc for inhibiting the firefly luciferase expression. In addition, to extend its utility for the establishment of inherited stable clones, we have also reconstructed this bidirectional expression system with the blasticidin S deaminase gene, an effective dominant drug resistance selectable marker, and examined both the selection and inhibition efficiencies in drug resistance and gene expression. Moreover, we have further demonstrated that this bidirectional expression system could efficiently co-regulate the functionally important genes, such as overexpression of tumor suppressor protein p53 and inhibition of anti-apoptotic protein Bcl-2 at the same time. In summary, the bidirectional expression vectors, pbiHsH1 and pbiMmH1, should provide a simple, convenient, and efficient novel tool for manipulating the gene function in mammalian cells.
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RNA interference (RNAi) is an evolutionarily conserved mechanism of posttranscriptional gene silencing induced by double-stranded RNAs (dsRNAs) [1,2]. The dsRNA is first cleaved into 21- to 23-nucleotide (nt) small interfering RNA (siRNA) duplexes with two symmetrical 2-nt 3′ overhangs by dsRNA-specific endonuclease Dicer [3,4]. The resulting siRNA in turn is incorporated efficiently into the RNA-induced silencing complex (RISC) that guides the enzymatic cleavage of homologous mRNA at the site where the antisense strand of siRNA is complementarily bound [5,6]. Primarily, RNAi appears to be involved in cellular defense mechanism and maintaining genomic integrity against viral infection and transposable elements [7,8], as well as in cellular gene regulation and chromosomal epigenetic control [9–11]. Currently, it has emerged as a practically used approach for reverse functional genomics

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[12–15] and in particular as an extremely powerful strategy for molecular therapeutics [16,17].

In mammalian cells, there are mainly two strategies in producing dsRNA by exogenous delivery of synthetic siRNA [17,18] or shRNA [19] and endogenous transcription of siRNA [20–22] or shRNA by expression system [23–26]. The silencing effect induced by synthetic dsRNA is transient and the target gene is reactive after a few days, but this approach is simple and efficient [17,27]. In contrast, the inhibition activity induced by expressed dsRNA can be easily manipulated in either inheritable or inducible manner, as well as this approach can be genetically combined with distinct experimental conditions, such as simultaneous expression of selectable marker either drug resistance or reporter gene, and specific gene [14,15,23,28]. In the endogenous expression approaches, the siRNA is formed by annealing two complementary sense and antisense RNAs that are transcribed by either mainly tandem or convergent dual RNA Pol III promoters [20–22], whereas the shRNA is derived by a single RNA Pol II or in particular Pol III promoter-driven small DNA template encoding the sense, a loop, and the antisense sequence [23–26]. The H1 and U6 from human and mouse are the most frequently used RNA Pol III promoters, since they transcribe from a well-defined start site and stop at a simple effective termination signal consisting of only five or six consecutive thymidine residues (Ts), therefore they are suitable for expression of the small RNA transcripts without 5′ capping and 3′ polyadenylation modification [29,30].

To explore broadly the applications of RNAi-based gene silencing, it is convenient to have an expression system that could transcribe both the protein-coding genes and shRNAs simultaneously. To fulfill this requisition, the expression vector contains either two independent expression cassettes or a bidirectional control system. In these two strategies, the bidirectional expression vector provides a simple and efficient tool for manipulating at least two distinct gene activities at the same time. There are a large number of conserved bidirectional gene pairs that tend to be co-expressed by sharing promoter sequences in mammalian genomes [31]. The PARP-2/RNase P bidirectional control promoter, one of the well-characterized bidirectional regulation systems, co-regulates the expression of both

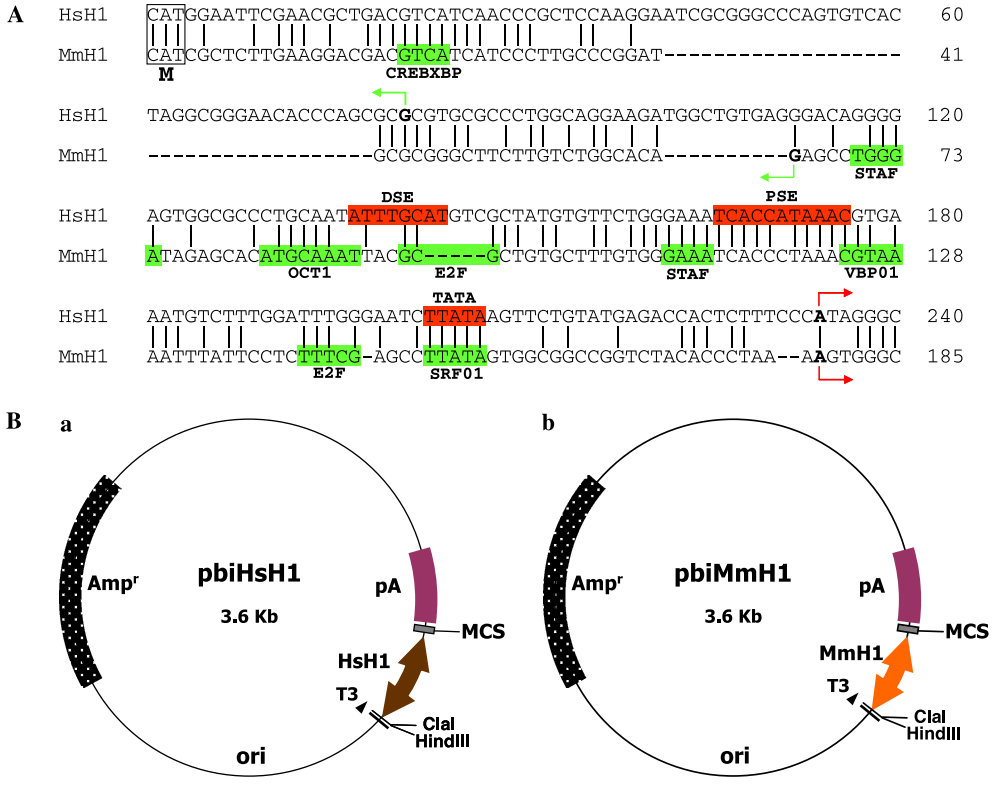

Fig. 1. Analysis of the PARP-2/RNase P bidirectional control promoter and structure of the bidirectional expression vectors, pbiHsH1 and pbiMmH1. (A) Sequence analysis of the PARP-2/RNase P bidirectional control promoters from human (HsH1) and mouse (MmH1). The HsH1 and MmH1 sequences are retrieved from Ame et al. [32] (Accession No. AF191547), Baer et al. [33] (Accession No. X16612), and Wu et al. [34]. The consensus binding sites and sequences for the essential transcription factors of Pol II and Pol III-dependent expression systems are highlighted in green and red, respectively. Transcription initial sites are indicated by green and red arrows on Pol II-controlled PARP-2 protein and Pol III-regulated RNase P RNA expression, respectively. **M** represents the amino acid methionine and CAT is the translation start codon on both human and mouse PARP-2 protein-coding regions. (B) Constructs of the bidirectional expression vectors, pbiHsH1 (a) and pbiMmH1 (b). These two vectors contained a conserved PARP-2/RNase P bidirectional control promoter from human (HsH1) or mouse (MmH1), a SV40 poly A (pA) signal for transcription termination, two specific restriction enzyme sites, *Cla*I and *Hin*dIII, for constructing the shRNA expression sequences, and a unique multiple cloning site (MCS) for cloning the genes of interest. MCS: 5′-*Eco*RI, *Pst*I, *Sma*I, *Xma*I, *Bam*HI, *Spe*I, *Xba*I, *Eaq*I, and *Not*I-3′.

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