Recyclable single-stranded DNA template for synthesis of siRNAs

Mussa M. Ali2,3, Demian Obregon3, Krishna C. Agrawal2, Mahmoud Mansour4 & Asim B. Abdel-Mageed1,2,*
1Departments of Urology and 2Pharmacology, Tulane University Health Sciences Center, New Orleans, Louisiana, 3Humesis Biotechnology Corporation, St. Petersburg, Florida, USA, 4Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama

RNA interference is a post-transcriptional silencing mechanism triggered by the bioavailability and/or exogenous introduction of double-stranded RNA (dsRNA) into cells. Here we describe a novel method for the synthesis of siRNA in a single vessel. The method employs in vitro transcription and a single-stranded DNA (ssDNA) template and design, which incorporates upon self-annealing, two promoters, two templates, and three loop regions. Using this method of synthesis we generated efficacious siRNAs designed to silence both exogenous and endogenous genes in mammalian cells. Due to its unique design the single-stranded template is easily amenable to adaptation for attachment to surface platforms for synthesis of siRNAs. A siRNA synthesis platform was generated using a 3' end-biotinylated ssDNA template tethered to a streptavidin coated surface that generates stable siRNAs under multiple cycles of production. Together these data demonstrate a unique and robust method for scalable siRNA synthesis with potential application in RNAi-based array systems. [BMB reports 2010; 43(11): 732-737]

INTRODUCTION

The introduction of dsRNA into the cell induces the sequence specific inhibition of the homologous gene. This mechanism of post-transcriptional silencing is referred to as RNA interference (RNAi) in animals, co-suppression in plants and quelling in fungi (1). The downstream effector silencing molecules, namely small interfering RNAs (siRNAs), are widely used to artificially trigger the 'knockdown' of the homologous gene (2). As the use of RNAi to silence genes of interest is becoming a routine tool in many experimental settings, inexpensive and simple methods (3) for generating the effector molecules, i.e., small interfering siRNAs, are needed. As a matter of empirical necessity, and to determine the optimal efficacy of the interfering molecule, more than one siRNAs are often designed and synthesized and tested before an efficacious siRNA compatible for silencing is generated (4).

Although chemically synthesized siRNAs are used in RNAi experiments, to date the use of synthetic siRNAs in RNAi experiments remain an expensive procedure. A less costly approach for siRNA generation employs a method in which oligonucleotide DNA templates, in vitro transcription (IVT), and other DNA and RNA modifying enzymes are used for the synthesis of the complementary strands (5-8). Whereas the costs associated with the transcription based methods are manageable, invariably these approaches require that the complementary sense and antisense strands be synthesized in separate vessels.

Despite the caveats associated with the various strategies for generating siRNAs (9-12), we investigated the feasibility of generating large quantities of siRNAs of defined length and sequence using a single-stranded DNA (ssDNA) designed to serve as a recyclable template for siRNA generation. Here we report a novel transcription-based siRNA synthesis method employing a self-annealing ssDNA template which generates two opposing promoters and two template regions to allow for a one-step co-transcription of the sense and antisense strands in a single vessel. Moreover, the ssDNA template is amenable to adaptation for attachment to surfaces to serve as a repeat-use platform for siRNA synthesis.

RESULTS AND DISCUSSION

ssDNA template design, siRNA synthesis and product analysis

We designed two 156 mer green fluorescent protein (GFP) and the human heterogeneous nuclear ribonucleoprotein H1 (hnRNAPH1) ssDNA templates which (when allowed to self-anneal) will generate two divergent T7 promoter regions, two template regions for the transcription of the sense and antisense strands and three loop regions to allow the single stranded DNA template to fold back (Fig. 1A). In order to facilitate T7 slippage and transcriptional termination we designed the third loop region to contain a string of A’s (Fig. 1A) (13). We employed a
robust system for synthesis of siRNAs (Fig. 1B). Briefly, the de-salted and lyophilized DNA templates (GeneLink) were re-suspended in nuclease free water at a final concentration of 100 pmol/μl. The templates (20 pmol/μl) were then allowed to form double stranded regions in an annealing buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl) by heating to 85°C for 2 min followed by cooling to room temperature for 30 min. Using standard IVT procedures, we generated 2 siRNAs designed to silence an exogenous GFP gene (Fig. 2A) and an endogenous hnRNPH1 gene (Fig. 2B). In order to determine the integrity of the in vitro transcribed GFP-siRNA and hnRNPH1-siRNA, aliquots of the IVT products were compared to a chemically syn-

http://bmbreports.org