BMB
reports

Recyclable single-stranded DNA template for synthesis of siRNAs

Mussa M. Ali[2,3], Demian Obregon[3], Krishna C. Agrawal[2], Mahmoud Mansour[4] & Asim B. Abdel-Mageed[1,2,]*

[1]Departments of Urology and [2]Pharmacology, Tulane University Health Sciences Center, New Orleans, Louisiana, [3]Humesis Biotechnology Corporation, St. Petersburg, Florida, USA, [4]Department 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

*Corresponding author. Tel: 504-988-2750; Fax: 504-988-5059; E-mail: amageed@tulane.edu
DOI 10.5483/BMBRep.2010.43.11.732

Reveived 2 September 2010 , Accepted 23 September 2010

Keywords: Recyclable, Single-stranded DNA template, siRNA, Synthesis

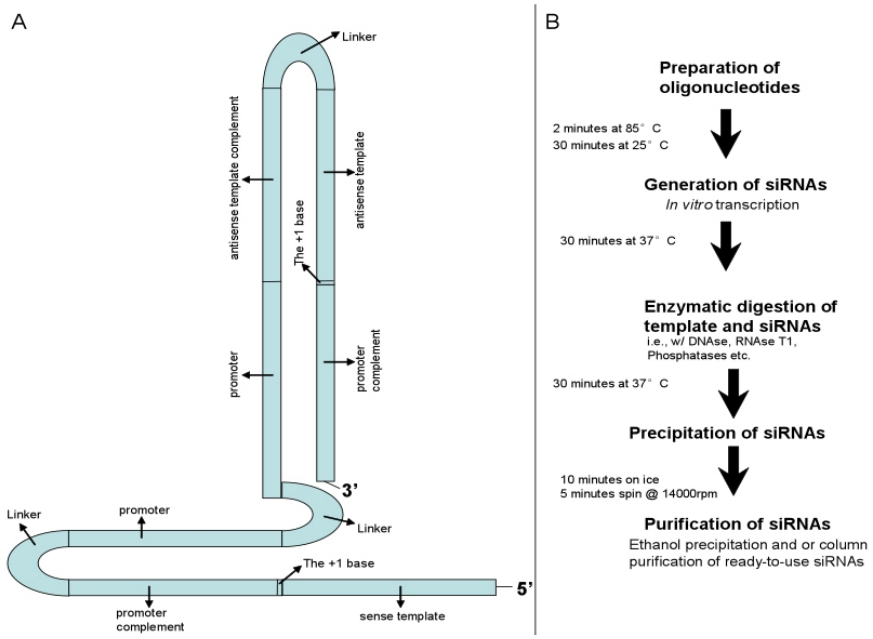

Fig. 1. Schematic and Flow chart: Single-stranded DNA template design and 'single-step' siRNA synthesis strategy. (A) a schematic of the template, loop and promoter regions of ssDNA. The 3'end of the template may include additional linker sequences to allow for efficient labeling or tethering to a surface platform. (B) Flow chart of the steps involved in the synthesis of siRNA molecules from ssDNA templates.

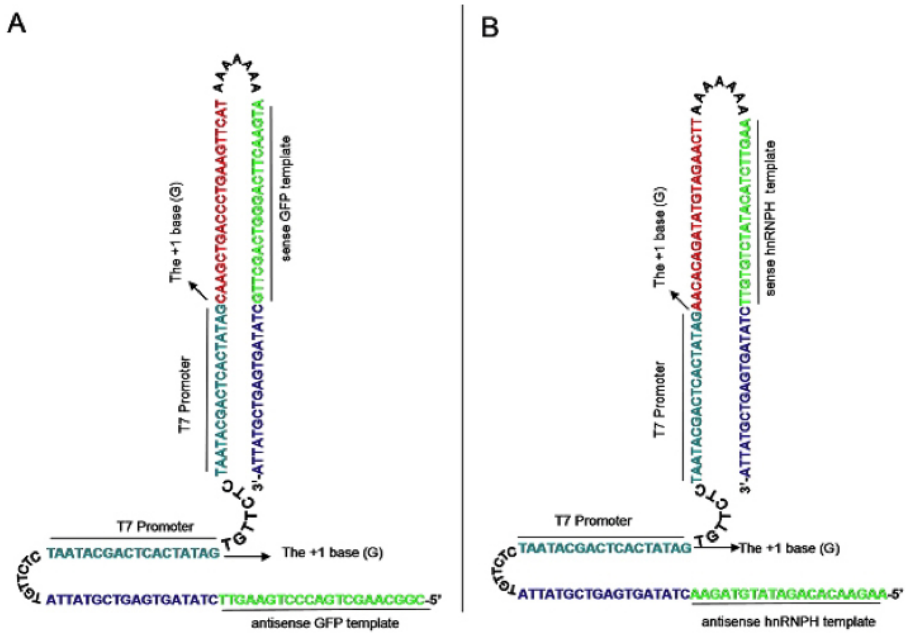

Fig. 2. Folded-back ssDNA sequences for the generation of GFP-siRNA and hnRNP H1. Diagrams of the siRNA-producing ssDNA templates illustrating predicted secondary structures after the annealing step. The 156 mer ssDNA template design allows for the generation of GFP-siRNA (A) and hnRNP H1 (B). Upon self annealing the ssDNA comprises of two divergent promoter regions, two template regions for the transcription of the sense and antisense strands and three loop regions.

robust system for synthesis of siRNAs (Fig. 1B). Briefly, the desalted and lyophilized DNA templates (GeneLink) were resuspended 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-

thesized 21 mer dsRNA molecular marker on a non-denaturing agarose gel. The *in vitro* synthesized siRNAs migrated similarly as that of the chemically synthesized marker (Fig. 3A). Additionally, we assessed the absorption properties of the generated siRNAs and found a 260/280 nm reading of greater than 1.9.

Functional efficacy of the GFP-siRNA in silencing an exogenous gene

The androgen sensitive and androgen independent prostate cancer MDA-PCa-2b cells were co-transfected with a 0.25 ug of GFP plasmid and 25 nM of siRNA-GFP or the siRNA-hnRNPH1. We observed no toxicity or change in viability of cells at these concentrations used and assessed by WST-8 (Alexis Biochemicals, San Diego, CA) (Fig. 3B). After 24 h, we then assessed the efficacy of the GFP-siRNA to silence GFP fluorescene. Next, we assessed the specificity and efficiency of unbound T7 transcribed siRNAs against GFP. The hnRNPH1-siRNA was used as a non-targeting control in GFP-transfected cells. The cells transfected with unbound siRNA showed a specific and potent silencing of GFP fluorescene (Fig. 3C-I), whereas GFP fluorescene was found to be unaffected in the control hnRNP H1-siRNA transfected cells (Fig. 3C-II). Therefore, our data demonstrated the efficacy of the unbound GFP-siRNA, generated using T7 polymerase, and the ssDNA template, which induced sequence specific inhibition of an exogenously introduced gene.

Functional analysis of the hnRNPH1-siRNA in silencing an endogenous gene

In addition to GFP, we tested the functional efficacy of unbound hnRNPH1-siRNA to silence an endogenous gene, hnRNP H1. To this end, MDA-PCa-2b cells were transfected with 25 nM of control GFP-siRNA or hnRNPH1-siRNA. Twenty four hours post-transfection, we then assayed the sequence specific degradation of the cognate hnRNP H1 mRNA by carrying out RT-PCR assays on the total mRNA extracted from GFP- siRNA and hnRNPH1-siRNA transfected MDA-PCa-2B cells. Our RT-PCR data showed that in the cells transfected with hnRNP H1-siRNA, the cognate mRNA was significantly reduced. However, in the house-keeping gene GAPDH, and in the control GFP-siRNA transfected cells, the hnRNPH1 mRNAs were unaltered (Fig. 4A). In addition, whole cell lysates in SDS sample buffer of transfected cells were fractionated onto 12% of polyacrylamide gels, and subsequently transferred to nitrocellulose using a semi-dry transfer approach. Target gene products were detected using anti-hnRNPH1 and anti-actin antibodies and ECL enhanced chemiluminescence technique. Our Western blot data showed that siRNA-hnRNP H1 mediated RNAi induced specific and potent inhibition of the expression of the endogenous hnRNP H1 protein (Fig. 4B). Moreover, immunoblot analysis of protein extracts of the control GFP-siRNA transfected cells showed that hnRNPH1 protein levels were unaltered (Fig. 4B). In both GFP-siRNA and hnRNPH1-siRNA transfected cells, the levels of the internal control actin proteins were unaltered (Fig. 4B). The results demonstrate that siRNA duplexes specifically inhibit the expression of an endogenous gene, hnRNPH1, which is highly expressed in MDA-PCa-2b cells.

Tethered ssDNA template-based generation of siRNAs

One of the obvious advantages of the ssDNA template used for generating siRNA in this study was the fact that the ssDNA is uni-molecular, and therefore easily amenable to tethering to various surfaces serving as a recyclable platform for siRNAs generation. To this end, 5 pmol per well of ssDNA GFP tem-

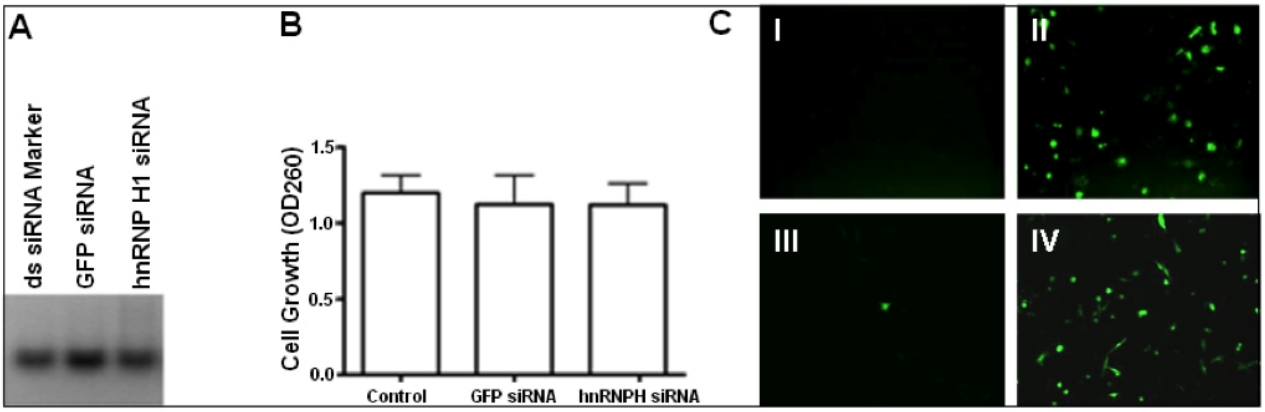

Fig. 3. Analysis and silencing efficiency of generated GFP siRNA products. (A) The *in vitro* transcribed GFP and hnRNPH1 siRNAs (1 ug/lane) were analyzed by migration through a non-denaturing 4% agarose gel and compared with a chemically synthesized 21 mer dsRNA marker. (B) Cytotoxicity assay of MDA-PCa-2b cells transfected with hnRNP H1 siRNA (WST-8 assay). (C) MDA-PCa-2b cells were co-transfected with a 0.25 ug of GFP siRNA plasmid and 25 nM of GFP siRNA (I and III) or the control hnRNPH1 (II and IV) siRNA generated from unbound (I and II) and bound (III and IV) ssDNA templates and subjected to fluorescence microscopy after 24 h.

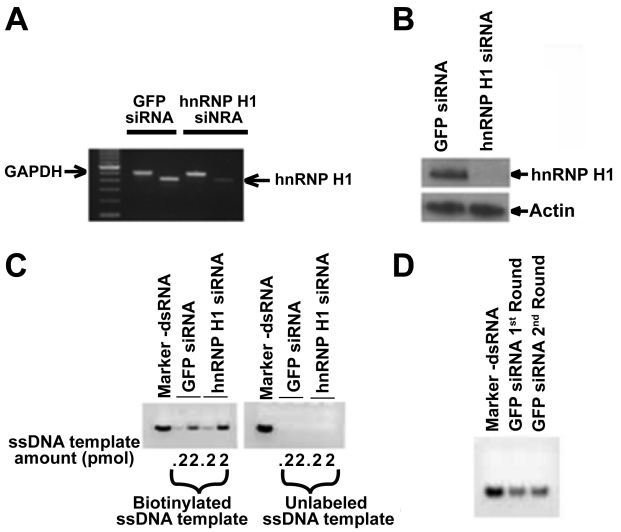

Fig. 4. Silencing efficacy and specificity of generated hnRNP H1 siRNAs. (A) RT-PCR analysis of hnRNP H1 silencing after transfection with either GFP or hnRNPH1 siRNA demonstrating efficacy and specificity. (B) Western blot analysis of hnRNPH1 expression after 25 nM hnRNPH1 and GFP (control) siRNA treatments. Actin was used as a control for protein loading and specificity of siRNA. (C) The 3′ end biotinylated ssDNA GFP template (0.2 and 2 pmol per well) were incubated in a streptavidin coated 96-well plate and after thorough washing to remove the unbound template, IVT was then carried out on the bound ssDNA template. The siRNA generated were then analyzed by electrophoresis on a 4% agarose gel and compared with 21 mer dsRNA marker. As a control, no IVT products were detected from the unlabelled GFP ssDNA template incubated wells. (D) Repeat use of the bound template platform for multiple rounds of siRNA generation. The analysis of GFP siRNAs generated after 1st and 2nd rounds of IVT on the bound ssDNA template from the same well. The well was thoroughly washed after each cycle of IVT.

plates were 3' end-labeled with biotin according to manufacturer's instruction. After labeling, the biotin-ssDNA GFP template was attached to streptavidin-coated 96-well plates. As a parallel control, we used 5 pmol per well of unlabelled ssDNA GFP template. After co-incubation of the 3' biotin end-labeled ssDNA template with the streptavidin coated surface, the wells were thoroughly rinsed 3 times. Using a standard IVT protocol, we generated GFP-siRNA for further analysis. The integrity of the product and the quality of siRNA duplex was verified using standard protocols. Our silencing analysis showed that the GFP-siRNAs generated, using the tethered biotin-ssDNA templates, were efficacious in silencing the expression of GFP (Fig. 3C-III) when compared to the control hnRNP H1 siRNA-transfected control (Fig. 3C-IV). To examine if the siRNA products are dependent on the amount of tethered template, various amounts (0.2 and 2 pmol per well) of the 3′ end biotinylated ssDNA GFP template were incubated in a streptavidin-coated 96-well plate. After thorough washing, IVT was then carried out on the bound ssDNA template. The siRNA generated positively correlated with the amount of tethered template when analyzed in comparison to a 21 mer dsRNA marker (Fig. 4C). As a control, no IVT products were detected from the unlabelled GFP ssDNA template incubated wells (Fig. 4C). To test the repeated use of the template, we generated additional GFP-siRNAs by recycling the streptavidin-biotin ssDNA template surface by yet another round of IVT (Fig. 4D).

Both chemically synthesized or DNA vector-based systems have been developed to express siRNA molecules for various applications. However, most, if not all, of the current approaches of chemically produced siRNA or transcriptional enzyme-based synthesis methods involve two separate steps for generation of the sense and antisense strands of the target gene (14). Production of siRNAs from a vector has predominantly been done through the transcription of a hairpin RNA that structurally mimics a miRNA precursor, allowing it to be processed into the effector siRNA inside the cell. More importantly, they are invariably not suitable for attachment to synthesize siRNAs in a recyclable platform. Here we describe a novel method for synthesis of siRNAs in a single vessel. This method of synthesis employs IVT and an ssDNA template encompassing, and when allowed to self-anneal generates double stranded promoter regions and three loop regions. Then, the sense and antisense strands can be simultaneously transcribed from the annealed DNAs. The newly synthesized RNA molecules self- anneal simultaneously into a stable siRNA molecules once transcribed *in vitro*. After enzymatic digestion, precipitation, and column purification, we demonstrate the efficacy of the generated siRNAs in silencing both exogenous and endogenous genes in mammalian cells. We also show that the T7 generated siRNAs neither exert toxicity in transfected cells nor provoke interferon response as shown by others (15). In comparison to current techniques, our novel approach represents a robust and efficient one-step tool for recyclable use of an ssDNA template for scalable synthesis of siRNA *in vitro*. Due to its unique design, we also found that our single-stranded template is easily amenable to adaptation for attachment to surface platforms. In the current study, we have generated and successfully tethered a 3' end-biotinylated ssDNA template to a streptavidin coated surface. These bound templates have the potential to be well suited recyclable platforms for high throughput synthesis of siRNAs.

MATERIALS AND METHODS

Design, synthesis and preparation of DNA templates

Two 156 mer self-annealing ssDNAs, which have two regions of template, two promoters and three loops, were designed to generate siRNA targeting GFP and hnRNPH1 (Fig. 1, 2). Desalted and lyophilized ssDNA templates were obtained from GeneLink (Hawthorne, NY). The template sequences (where sequences are in bold face and the loop regions are underlined) for GFP and hnRNPH1 siRNA synthesis are as fol-

lows; (GFP, 5'CGGCAAGCTGACCCTGAAGTTCTATAGTGAG TCGTATTATGTTCTCTAATACGACTCACTATAGTGTTCTCTA ATACGACTCACTATAGCAAGCTGACCCTGAAGTTCATAAA AAAATGAACTTCAGGGTCAGCTTGCTATAGTGA GTCGTAT T A3'; hnRNPH1, 5'AAGAACACAGATATGTAGAACTATAGT GAGTCGTATTATGTTCTCTAATACGACTCACTATAGTGTTC TCTAATACGACTCACTATAGAACACAGATATGTAGAACTTA AAAAAAAAGTTCTACATATCTGTGTTCTATAGTGAGTCGTA TTA3'. The templates were suspended in nuclease free water to a final concentration of 100 pmoles/μl. An aliquot of the DNA templates were then allowed to self-anneal in an annealing buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl) at a final concentration of 20 pmol/μl by heating to 85°C for 2 min followed by slow cooling to room temperature for 30 min.

In vitro transcription

T7 polymerase driven IVT on the ssDNA templates were performed according to previously described reports (16). Briefly, the following were added into a single vessel: transcription buffer (42 mM Tris-HCl pH 7.9, 11 mM NaCl, 4.5 mM $MgCl_2$, 2.5 mM spermidine, and 11 mM DTT), 0.15 units yeast pyrophosphatase, 2 mM rNTP, 40 U RNase inhibiting peptide and 100 units T7 RNA polymerase, 20 pmol self-annealed ssDNA template. Next, mixtures were incubated at 37°C for 30 min, after which 1 U of DNAse-I was added. Then, the mixtures were in cubated incubated at 37°C for 30 min to remove template DNA. In conditions in which the T7 transcription start nucleotide (G) is not part of the gene, we added RNAseT1 in order to cleave off this nucleotide. The generated siRNA duplexes were precipitated by addition of ice cold 0.1 X volume of 3M sodium acetate (pH 5.1), and 1 X volume of isopropanol and allowed to incubate for 10 min on ice. The IVT products were then centrifuged at maximum speed (14,000 rpm) for 10 min. The pellets were washed twice with 75% ethanol and air dried, then resuspended in pure water by heating at 55°C for 10 min. The products were further purified through a Micro Bio-Spin 6 Column (Bio-Rad). The integrity and size of siRNA duplexes were assessed by standard gel-electrophoresis and 260/280 nm absorption with a spectrophotometer. The siRNA duplexes were then frozen at −80°C until used.

Cell culture and viability assay

The androgen sensitive and androgen independent prostate cancer MDA-PCa-2b cells were purchased from American Type Tissue Culture (ATCC, Manassas, VA) and were maintained at 37°C in 5% CO_2 in a BRFF-HPC1 medium supplemented with 10% FBS, penicillin (100 IU/ml), and streptomycin (100 μg/ml). The bone metastasis-derived MDA-PCa-2b cell line overexpressed the nuclear matrix hnRNPH1 gene. The viability of siRNA-transfected cells was monitored with WST-8 cell counting kit (Alexis Biochemicals, San Diego, CA) as we described previously (17).

Selection of efficacious siRNA sequences for GFP and hnRNP-H1 gene silencing

Three chemically synthesized SmartPool sequences with high siRNA efficiency were designed for hnRNPH1 gene (GenBank accession #NM_005520) using special algorithm software at siDesigne Center (Dharmacon, Inc). The efficiency of gene silencing and cytotoxicity of individual siRNA sequences were examined in MDA-PCa-2b cells as described (2, 4). Validated siRNA sequences with high silencing efficiency for hnRNPH1 (sense 5'-GAACACAGAUAUGUAGAACUU-3' and antisense 5'-GUUCUACAUAU CUGUGUUCUU-3') and GFP (18) were used in our proof-of-principle studies. Potent and specific silencing of target genes were achieved with 25 nM concentrations of T7 generated siRNA duplexes in all our RNAi experiments.

Transfection

Cells ($1\text{-}3\times10^5$ cells/ml) were cultured in 12-well plates overnight. To achieve a 90% transfection efficiency, transfection mixture consisting of 2 ml serum-free Opti-MEM I (Gibco-BRL), 80 μl Lipofectamine 2,000 (Invitrogen) and 15 μl of the RNAse inhibitor at 20 U/μl (Ambion) were combined with siRNA (hnRNPH-1 or GFP) and incubated for 30 min at 25°C. DMEM plus 10% FBS (Invitrogen) were added to bring the final siRNA concentration to 25 nM. The culture medium was then removed and replaced with 95 μl of transfection mixture and cells were incubated overnight for 18 h.

RT-PCR analysis

Silencing of the target gene mRNAs was determined using RT-PCR as we previously described (19, 20). Briefly, RNA of control and siRNA-silenced cells was extracted by RNeasy kit (Qiagen), reverse transcribed, and subsequently amplified using hnRNP-H1 (20), GFP and GAPDH specific primers. The GFP primers (forward, 5'GGAGAGGGTGAAGGTGATGC 3'; reverse, 5'TGTTCTTGTAGTTCCC GTCATC 3') were designed in our laboratory and were assessed for their ability to amplify a single amplicon, a sequence of which is consistent with the GFP cDNA. PCR products were UV visualized and quantified by demsitometric analysis (Bio-Rad, model 700).

Western blot analysis

Immunoblot blot analysis was performed as described by Abdel-Mageed *et al*. (21). Briefly, whole cell lysates in a SDS sample buffer of transfected cells were fractionated onto 12% of polyacrylamide gels, and subsequently transferred to nitrocellulose using a semi-dry transfer approach. Target gene product was detected using anti hnRNP-H1 (Novus Biologicals) antibodies and blots were developed using secondary antibodies and ECL enhanced chemiluminescence technique as per manufacturer's instructions (Amersham Pharmacia).

GFP fluorescence scanning

Cells were co-transfected with 0.25 μg of GFP plasmid and 25

nM of GFP siRNA or hnRNP-HI siRNA as control. The cells were then scanned for GFP fluorescence 24 h later (Nikon).

3'End-biotinylation of ssDNA template and attachment to a streptavidin-coated surface

ssDNA templates for GFP 3' were end-labeled with biotin according to manufacturer's instructions (Pierce, Rockford, IL). Two pmols per well of biotin labeled ssDNA templates were incubated onto streptavidin coated 96-well plate. As a control, an equimolar amount of unlabelled (non-biotinylated) ssDNA was incubated in the streptavidin-coated wells. Unbound templates were rinsed off 3 x in annealing buffer (10 mM Tris-HCl pH 7.5, 50 mM NaCl). The biotin-ssDNA streptavidin wells were subsequently used for IVT and generation of the siRNA duplexes. The IVT products were precipitated and column purified as described above (see *Materials and Methods*, IVT section). No appreciable IVT products were detected from the control wells, in which unlabelled ssDNA was used. IVT products generated using biotin-streptavidin tethered ssDNA were analyzed by absorption spectrophotometry at 260/280 nm. Additionally, the generated siRNA duplexes were run on a non-denaturing gel and showed the same mobility as a chemically synthesized 21 mer dsRNA marker.

Acknowledgements

This work is partially supported by a grant from American Cancer Society (TURSG CCE-101610).

REFERENCES

1. Tomari, Y. and Zamore, P. D. (2005) Perspective: machines for RNAi. *Genes Dev.* **15**, R61-R64.
2. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl, T. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **441**, 494-498.
3. Sohail, M., Doran, G., Riedemann, J., Macaulay, V. and Southern, E. M. (2003) A simple and cost-effective method for producing small interfering RNAs with high efficacy. *Nucleic. Acids. Res.* **31**, e38.
4. Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W. S. and Khvorova, A. (2004) Rational siRNA design for RNA interference. *Nature Biotechnol.* **3**, 326-330.
5. Donzé, O. and Picard, D. (2002) RNA interference in mammalian cells using siRNAs synthesized with T7 RNA polymerase. *Nucleic. Acids. Res.* **30**, e46.
6. Oleinikov, A. V., Zhao, J. and Gray, M. D. (2005) RNA interference by mixtures of siRNAs prepared using custom oligonucleotide arrays. *Nucleic. Acids. Res.* **33**, e92.
7. Yu, J. Y., DeRuiter, S. L. and Turner, D. L. (2002) RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A* **99**, 6047-6052.
8. Luo, B., Heard, A. D. and Lodish, H. F. (2004) Small interfering RNA production by enzymatic engineering of DNA (SPEED). *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5494-5499.
9. Rose, S. D., Kim, D. H., Amarzguioui, M., Heidel, J. D., Collingwood, M. A., Davis, M. E., Rossi, J. J. and Behlke, M. A. (2005) Functional polarity is introduced by dicer processing of short substrate RNAs. *Nucleic. Acids. Res.* **33**, 4140-4156.
10. Jiang, M., Arzumanov, A. A., Gait, M. J., Milner, J. A. (2005) Bi-functional siRNA construct induces RNA interference and also primes PCR amplification for its own quantification. *Nucleic. Acids. Res.* **33**, e151.
11. Castanotto, D. and Rossi, J. J. (2004) Construction and transfection of PCR products expressing siRNAs or shRNAs in mammalian cells. *Methods Mol. Biol.* **252**, 509-514.
12. Kim, J., Kim, H., Lee, Y., Yang, K., Byun, S. and Han, K. A. (2006) Simple and economical short-oligonucleotide-based approach to shRNA generation. *J. Biochem. Mol. Biol.* **39**, 329-334.
13. MacDonald, L. E., Zhou, Y. and McAllister, W. T. (1993) Termination and slippage by bacteriophage T7 RNA polymerase. *J. Mol. Biol.* **232**, 1030-1047.
14. Donzé, O. and Picard, D. (2002) RNA interference in mammalian cells using siRNAs synthesized with T7 RNA polymerase. *Nucleic. Acids. Res.* **30**, e46.
15. Strat, A., Gao, L., Utsuki, T., Cheng, B., Nuthalapaty, S. Mathis, J. M., Odaka, Y. and Giordano, T. (2006) Specific and nontoxic silencing in mammalian cells with expressed long dsRNAs. *Nucleic. Acids. Res.* **34**, 3803-3810.
16. Milligan, J. F., Groebe, D. R., Witherell, G. W. and Uhlenbeck, O. C. (1987) Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic. Acids. Res.* **15**, 8783-8798.
17. Davis, R., Jia, D., Cinar, B., Sikka, S. C., Moparty, K., Zhau, H. E., Chung, L. W., Agrawal, K. C. and Abdel-Mageed, A. B. (2003) Functional androgen receptor confers sensitization of androgen independent prostate cancer cells to anticancer therapy via caspase activation. *Biochem. Biophy. Res. Commun.* **309**, 937-945.
18. Caplen, N. J., Parrish, S., Imani, F., Fire, A. and Morgan, R. A. (2001) Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9742-9747.
19. Abdel-Mageed, A. B. and Agrawal, K. C. (1998) Activation of nuclear factor Kappa B: potential role in metallothionein-mediated mitogenic response. *Cancer Res.* **58**, 2335-2338.
20. Honore, B., Vorum, H. and Baandrup, U. (1999) hnRNPs H, H' and F behave differently with respect to posttranslational cleavage and subcellular localization. *FEBS Lett.* **456**, 274-280.
21. Abdel-Mageed, A. B. and Agrawal, K. C. (1997) Antisense down-regulation of metallothionein induces growth arrest and apoptosis in human breast carcinoma cells. *Cancer Gene Ther.* **4**, 199-207.