Available online at www.sciencedirect.com

ScienceDirect

Developmental and Comparative Immunology 31 (2007) 340–346

Developmental & Comparative Immunology

www.elsevier.com/locate/devcompimm

Histone H2A as a transfection agent in crayfish hematopoietic tissue cells

Haipeng Liu, Irene Söderhäll*

Department of Comparative Physiology, Uppsala University, Norbyvägen 18A, Uppsala 752 36, Sweden

Received 20 June 2006; received in revised form 17 July 2006; accepted 19 July 2006
Available online 11 September 2006

Abstract

We report a novel and highly efficient dsRNA transfection system based on one of the nuclear proteins, namely, histone H2A. RT-PCR semi-quantitative analysis of silencing target gene shows that the transfection efficiency of histone H2A is higher than Effectene or liposome-based transfection systems. Importantly, the high efficiency of histone H2A was associated with very low toxicity to the transfected crayfish hematopoietic tissue (Hpt) cells. The non-toxicity, effectiveness and specificity of histone H2A as a transfection agent provides a cheap, simple, highly efficient and reproducible gene delivery system, particularly for the sensitive cell cultures of crustacean animals such as crayfish and shrimp.

Keywords: Crayfish; Hematopoietic tissue cells; Histone H2A; RNA interference; Transfection

1. Introduction

The unique features of DNA-condensing capacity and nuclear localization signals (NLSs) make nuclear proteins, such as histones [1] and high-mobility group (HMG) proteins [2,3], excellent gene vectors to deliver DNA into cells. Histone H2A, one of the histone octamers that packages genomic DNA in eukaryotes, can mediate transfection efficiently [4] and it has been used for the cytokine gene transfer of IL-2 and a single chain IL-12 (scIL-12) to induce efficient antitumor responses in murine neuroblastoma [5]. Histone H2A has also successfully been used to intensify the efficiency of retroviral transfection [6]. The DNA-delivery activity of histone H2A was reported to be mediated by two mechanisms: (i) electrostatically driven DNA binding and condensation by histone and (ii) nuclear import of these histone H2A · DNA polyplexes via NLSs in the histone H2A [7]. So far, histone or histone-like proteins have been demonstrated as effective mediators of transfection, usually with better transfection results as compared to liposome-based transfection systems due to a better transfection efficiency and lower toxicity to the transfected cells by condensing and compacting DNA through interactions with the negatively charged sugar-phosphate backbone [3,8–10]. Fritz et al. demonstrated an effective in vitro gene transfer of histone H1 and a modified H1 molecule with a SV40 nucleophilic signal as the DNA carrier

Abbreviations: ALF, anti-lipopolysaccharide factor; CPBS, crayfish phosphate buffer saline; HMG, high-mobility group; Hpt, hematopoietic tissue; NLSs, nuclear localization signals; PAPI I, *Pacifastacus* proteinase inhibitor I

*Corresponding author. Tel.: +46 18 4712817; fax: +46 18 4716425.
E-mail address: irene.soderhall@ebc.uu.se (I. Söderhäll).

0145-305X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2006.07.007

[11]. Demirhan et al. reported that histone H3 and H4, but neither histone H1 nor histone 2A, were effective in the delivery of HIV-1 *tat* gene into Jurkat cells [12]. A recombinant histone H1 · 4F was shown to deliver DNA, dsRNA and siRNA into a variety of different mammalian cell lines as well as the insect Sua 4.0-*Anopheles gambiae* cells [13]. Moreover, histones H2A and H4 were recently shown for the first time to deliver covalently attached BSA molecules into plant cells [2].

In this paper, we show that transient transfection using a histone H2A-mediated gene delivery system led to high RNA silencing efficiency of the anti-lipopolysaccharide factor (ALF) and the *Pacifastacus* proteinase inhibitor I (PAPI I), two genes present in crayfish hematopoietic tissue (Hpt) cells.

2. Materials and methods

2.1. Animals

Freshwater crayfish, *Pacifastacus leniusculus*, were purchased from Nils Fors, Torsång at Lake Vättern, Sweden. Healthy intermolt male crayfish for Hpt cell cultures were maintained in aerated tap water at 10 °C.

2.2. Crayfish Hpt cell culture and maintenance

The Hpt cells were isolated from freshwater crayfish, *P. leniusculus*, as described by Söderhäll et al.[14]. Briefly, the Hpt was dissected from the dorsal side of the stomach and washed with CPBS (crayfish phosphate buffer saline: 10 mM Na_2HPO_4, 10 mM KH_2PO_4, 150 mM NaCl, 10 μM $CaCl_2$ and 10 μM $MnCl_2$, pH 6.8) and then incubated in 500 μl of 0.1% collagenase (type I and IV) (Sigma, Steinheim, Germany) in CPBS at room temperature for 45 min to dissociate the Hpt cells. The separated cells were washed twice with CPBS by spinning down at $2500 \times g$ for 5 min at room temperature. The cell pellet was then re-suspended in a modified L-15 medium [15] and subsequently seeded at a density of 5×10^4 cells/150 μl in 96-well plates. Hpt cells were supplemented with a crude astakine preparation [15] after about 30 min attachment at room temperature and 1/3 of the culture medium was changed every second day.

2.3. Generation of dsRNA

Oligonucleotide primers were designed to amplify an ALF 541 bp and a PAPI I 532 bp fragment, respectively, from a forward subtracted *P. leniusculus* hemocyte library (http://www.fu.uu.se/jamfys/pub3.html.), and they were incorporated with T7 promoter sequences (italics) at the 5′ ends: 107 +5′*TAATACGACTCACTATAGGG*ACGTGGGTACTAGTGA3′ and 647-5′*TAATACGACTCACTATAGGG*TCCAGGAAGATGCGACTACCA3′ for ALF cDNA; 309 + 5′*TAATACGACTCACTATAGGG*GCAACCTGTGCGCTCTAAGGATAG3′ and 840-5′*TAATACGACTCACTATAGGG*GAGTCCATGACGTGAATCTTCGTG3′ for PAPI I cDNA. A control 657 bp template was generated by PCR using primers specific for portions of the GFP gene from pd2EGFP-1 vector (Clontech, Palo Alto, CA, USA), and the primers were: 63 + 5′*TAATACGACTCACTATAGGG*CGACGTAAACGGCCACAAGT3′, 719-5′*TAATACGACTCACTATAGGG*TTCTTGTACAGCTCGTCCATGC3′. To generate dsRNA, PCR products purified with gel extraction (Qiagen, Hilden, Germany) were used as templates for in vitro transcription using the MegaScript kit (Ambion, Austin, TX, USA), and the dsRNAs were purified with Trizol® LS Reagent (Invitrogen, Carlsbad, CA, USA).

2.4. RNA interference in Hpt cell cultures using histone H2A as a transfection reagent

Based on the feature of histone H2A as a nucleic acid carrier vector, 4 μl dsRNA (250 ng/μl) was mixed with 3 μl calf histone H2A (histone from calf thymus, Type II-A, 1 mg/ml dissolved in modified L-15 medium) (Sigma, Steinheim, Germany) for each well of the Hpt cell culture and incubated for 5–10 min at room temperature and then followed by adding and mixing with 20 μl modified L-15 medium [15]. This was then added to 3 day old Hpt cell cultures. After incubation for 12 h at 16 °C, this medium was replaced with 150 μl medium together with 5 μl crude astakine preparation [15] and incubated for 2, 5 and 9 days, respectively, followed by the isolation of total RNA from that cell cultures. One-third of the total volume of medium was changed every second day during incubation of the Hpt cell cultures.

A series of naked dsRNA (100–1000 ng) were inoculated into Hpt cell cultures followed by isolation of total RNA 3 days post-dsRNA inoculation

to determine the RNA interference efficiency without addition of any transfection reagent, since naked dsRNA can be taken up by the cells directly.

To optimize the minimum amount of dsRNA for efficient RNA silencing, different amounts of dsRNA (100–1000 ng) were used to transfect the Hpt cell cultures using histone H2A as a transfection reagent. Isolation of that RNA for RT-PCR was performed 3 days after transfection.

2.5. RNA silencing efficiency and evaluation of toxicity to crayfish Hpt cells by different transfection reagents

Three day old crayfish Hpt cell cultures were transfected with ALF dsRNA or PAPI I dsRNA (200 ng/well) using different transfection agents such as histone H2A, Effectene® transfection reagent (Qiagen, Hilden, Germany), Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA), Lipofectin® reagent (Invitrogen, Carlsbad, CA, USA) or calcium phosphate transfection kit (Invitrogen, Carlsbad, CA, USA) following the manufactures' instructions for each transfection agent. The cell culture medium was replaced with fresh medium 12 h post-transfection. Total RNA from the dsRNA-transfected Hpt cell cultures was isolated 3 days post-transfection and was prepared for RT-PCR detection. Transfected Hpt cells were also prepared for cell viability determination by adding 5 μl/well of 0.4% trypan blue solution (Sigma, Steinheim, Germany) to the dsRNA silenced Hpt cell cultures 12 h post-dsRNA transfection and viable cell counting was carried out within 10 min after inoculation with the trypan blue solution.

2.6. Isolation of total RNA and semi-quantitative RT-PCR

Total RNAs were isolated from Hpt cell cultures using GenElute™ Mammalian Total RNA Miniprep Kit (Sigma, Steinheim, Germany), followed by RNase free DNase I (Ambion, Austin, TX, USA) treatment. Equal amount of total RNA was used for cDNA synthesis with ThermoScript™ (Invitrogen, Carlsbad, CA, USA) according to the manufacture's instructions and analyzed for expression of crayfish ALF and PAPI I genes using the following primers: 48 + 5′TCCGGAATCTCCTGACAACC3′ and 498-5′TGCGAAGATCTCGGAACTAGGA3′ for ALF; 139 + 5′TGACCTCAAGACGTATGGCAACAG3′ and 597-5′GTCCCGCACACAGGATCGTATT3′ for PAPI I. Crayfish 40s ribosomal protein primers were used in all PCR experiments as internal control for semi-quantification. The PCR program used was as follows: 94 °C, 3 min, followed by 29 cycles of 94 °C for 30s, 60 °C for 30s, 72 °C for 40s. All PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide.

3. Results

We first tested whether histone H2A worked as a dsRNA transfection reagent in crayfish Hpt cell cultures. As shown in Fig. 1, dsRNA transfection into crayfish Hpt cells mediated by histone H2A resulted in the reduction of crayfish ALF transcript 2 days after transfection and almost no transcript could be observed at the 5th and 9th day post-dsRNA transfection. Moreover, there was no difference in the amount of ALF transcript in the GFP dsRNA-transfected cells, suggesting that the ALF dsRNA silencing was sequence specific and efficient in crayfish Hpt cells.

Since naked dsRNA could be taken up by cells without any transfection reagent [16,17], we added naked ALF dsRNA directly into the crayfish Hpt cell cultures without addition of any transfection reagent. As shown in Fig. 2A, naked dsRNA could be taken up by Hpt cells and could induce RNA silencing effects on the cognate RNAs. However, the silencing efficiency was much lower than dsRNA transfection mediated by histone H2A (Fig. 2B), which showed that a transfection agent was required for obtaining a complete RNA silencing effect.

To optimize the minimum amount of dsRNA for an efficient RNA silencing, a series of ALF dsRNA

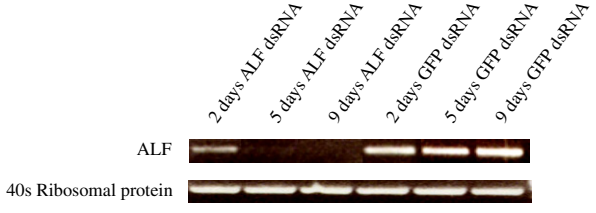

Fig. 1. Specificity of ALF dsRNA silencing effect on crayfish Hpt cells mediated by histone H2A. ALF or GFP dsRNA (1 μg/well) was incubated with 16.5 μg/ml of histone H2A for 10 min and then added to 3 day old crayfish Hpt cell cultures. Total RNA for semi-quantitative RT-PCR detection was isolated from the transfected Hpt cells after 2, 5, and 9 days, respectively.

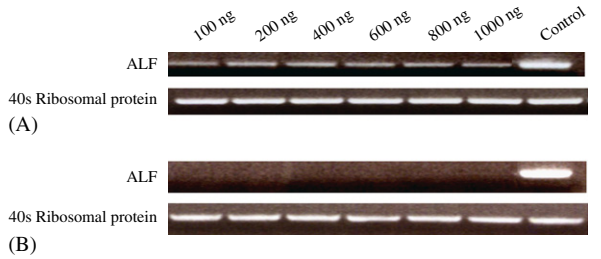

Fig. 2. (A) ALF silencing effects on crayfish Hpt cells by naked ALF dsRNA. Three days old crayfish Hpt cell cultures were inoculated with a series of ALF dsRNA (100–1000 ng/well). Total RNA was prepared for semi-quantitative RT-PCR detection 3 days post-dsRNA inoculation. Untreated cells were used as control. (B) Optimization of ALF dsRNA amount for efficient gene silencing effect on crayfish Hpt cells mediated by histone H2A. A series of ALF dsRNA (100–1000 ng/well) were transfected into 3 day old crayfish Hpt cell cultures mediated by 16.5 μg/ml of histone H2A. Total RNA from transfected Hpt cells was prepared 3 days post-dsRNA transfection for semi-quantitative RT-PCR detection. Untreated cells were used as control.

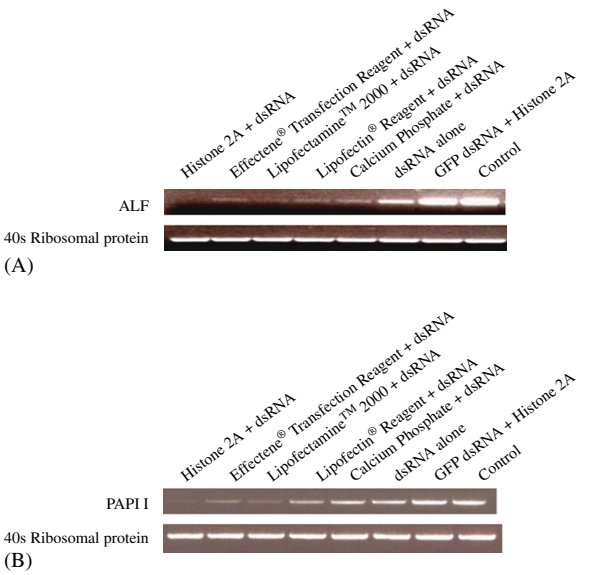

Fig. 3. (A) ALF dsRNA silencing effects on crayfish Hpt cells mediated by different transfection methods. ALF dsRNA (200 ng/well) was transfected into 3 day old crayfish Hpt cell cultures using histone H2A, Effectene® transfection reagent, Lipofectamine™ 2000, Lipofectin® reagent and calcium phosphate transfection kit, respectively, following our protocol or the manufacturers' instructions. Semi-quantitative RT-PCR were performed with total RNA isolated from the transfected Hpt cells 3 days post-dsRNA transfection. ALF dsRNA alone, GFP dsRNA transfected with histone H2A and untreated cells were used as controls. (B) PAPI I dsRNA silencing effects on crayfish Hpt cells mediated by different transfection methods. PAPI I dsRNA (200 ng/well) was transfected into 3 day old crayfish Hpt cell cultures mediated by different transfection reagents and total RNA was isolated for semi-quantitative RT-PCR as described above.

was used to transfect Hpt cells mixed with histone H2A as a transfection reagent. As shown in Fig. 2B the ALF gene expression could be silenced completely with as low as 100 ng of ALF dsRNA in combination with histone H2A.

Additionally, histone H2A-mediated transfection was also compared with Effectene or liposome-based transfection methods. The results showed that the silencing efficiency of histone H2A method was higher than the other commercial transfection methods. This effect was demonstrated by ALF as well as PAPI I gene silencing, two genes that are constitutively expressed in crayfish Hpt cells (Fig. 3A-B). Light microscopic examination of transfected cells with either Effectene or liposome-based transfection showed strong morphological alterations and cell disruption. Moreover, considerable cell death was observed in Effectene-transfected cells (Fig. 4). Besides the higher silencing efficiency, the use of histone H2A transfection did not affect the crayfish Hpt cell morphology or viability. No cell morphologic difference was observed between histone H2A-transfected cells and control cells suggesting that there is no toxic effect caused by using histone H2A as a transfection reagent (Fig. 4).

In order to evaluate toxicity, we monitored the number of viable crayfish Hpt cells 12 h post-incubation with different transfection reagents. Different cell viabilities were obtained among these transfection methods, which were 96.9%, 79.4%, 92.1%, 90.2% and 92.7% for histone H2A, Effectene® transfection reagent, Lipofectamin™ 2000, Lipofectin® reagent and calcium phosphate transfection kit, respectively, (Fig. 5). Notably was that the histone H2A transfection method was clearly shown to be non-toxic (96.9% cell viability) to the cells as compared to the control cells (97.3% cell viability).

4. Discussion

Histone H2A is used as a vector to achieve high transfection efficiency by high DNA packaging capacity and its NLSs. A nuclear localization sequence has been found in the amino terminal tail of histone H2A [1]. The DNA is condensed by interaction with histone H2A, as a transfection reagent, which provides significant advantages over

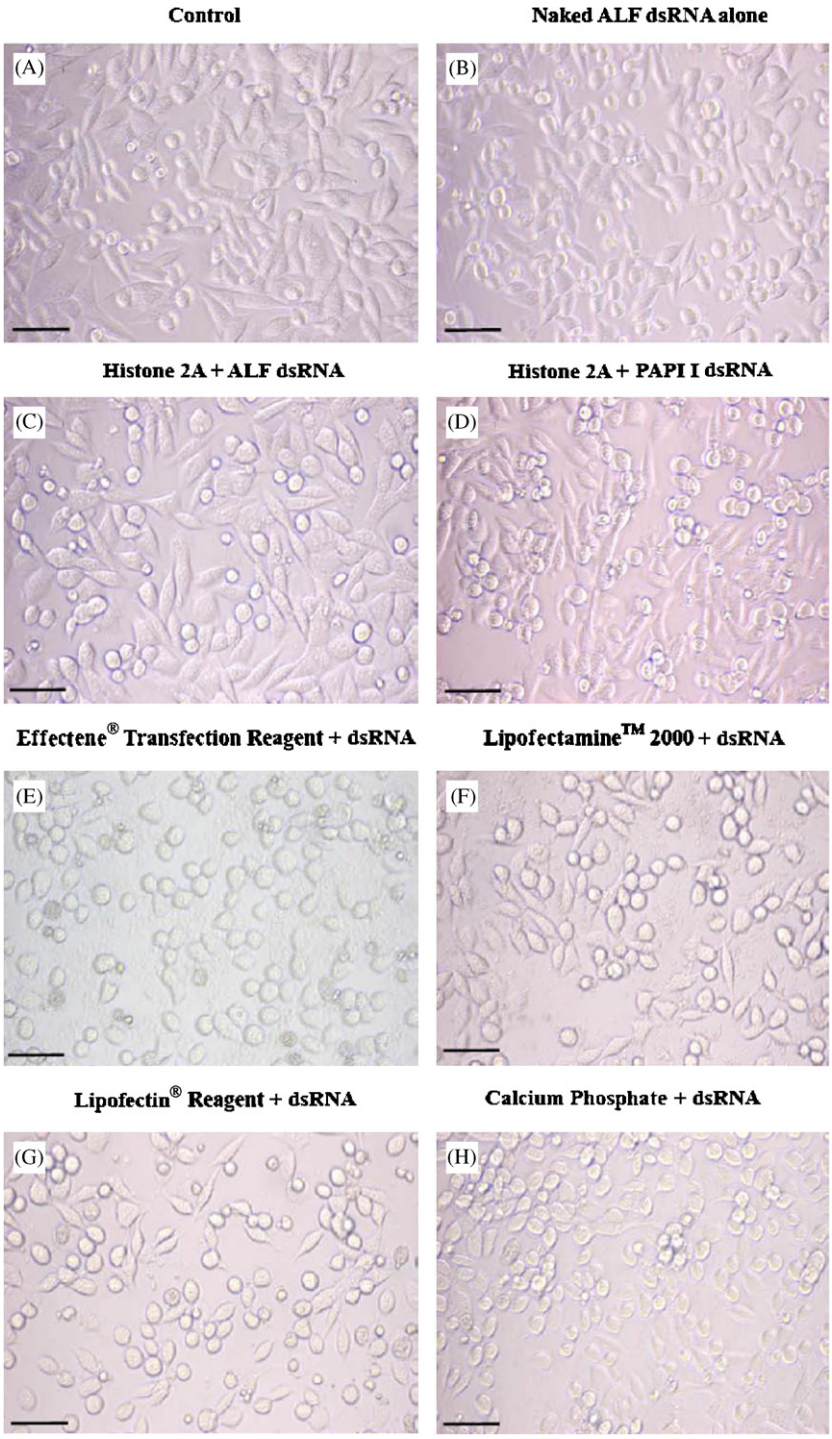

Fig. 4. Morphologic alteration of crayfish Hpt cells caused by different transfection reagents. Three day old crayfish Hpt cell cultures were transfected with 200 ng/well of ALF dsRNA or PAPI I dsRNA mediated by histone H2A, Effectene® transfection reagent, Lipofectamine™ 2000, Lipofectin® reagent, calcium phosphate transfection kit, or naked dsRNA inoculation alone, respectively. Light microscopic observations were done 12 h post-dsRNA transfection. Bars, 20 μm. (A) Untreated control cells; (B) naked ALF dsRNA alone; (C) ALF dsRNA transfection with histone H2A; (D) PAPI I dsRNA transfection with histone H2A; (E) ALF dsRNA transfection with Effectene® transfection reagent; F, ALF dsRNA transfection with Lipofectamine™ 2000; G, ALF dsRNA transfection with Lipofectin® reagent; H, ALF dsRNA transfection with calcium phosphate transfection kit.

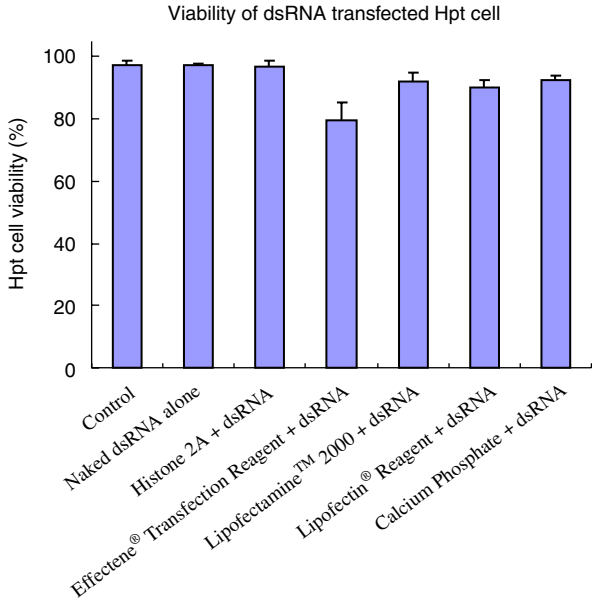

Fig. 5. Viability of dsRNA-transfected crayfish Hpt cells mediated by different transfection reagents. Crayfish Hpt cells were transfected with 200 ng/well of ALF dsRNA or PAPI I dsRNA as mentioned above. Five microliter of 0.4% trypan blue solution was added to the transfected cells 12 h post-transfection. The viable cell counting was carried out within 10 min following the inoculation of trypan blue solution. Untreated cells were used as a control treatment. The experiment has been repeated 4 times and the data represent mean of four replicates and the error bars indicate standard deviation.

many liposome/Effectene based reagents and other transfection methods. Furthermore, histone H2A is highly suitable for primary cells or sensitive cell lines due to its lower toxicity as a transfection reagent. Our findings here prove that delivery of dsRNA by histone H2A is capable of mediating specific gene silencing of two constitutively expressed genes in crayfish Hpt cells. In addition, in our hands, histone H2A-based transfection system also worked well in silencing of the cognate RNAs through delivering dsRNA into *Drosophila* mbn2 cells (data not shown). Hereby, our data show that calf thymus histone H2A is an effective mediator of transfection without any additional agents, which is consistent with a previous study showing similar transfection results in mammalian cells [7].

Histones, H3 and H4, alone with histone H2A and H2B, which bind and package DNA, are known to be involved in the regulation of gene expression [18–20] and these proteins also exert extracellular functions as histone H1 could mediate the binding of thyroglobulin to the cell surface of mouse macrophages [21,22]. Histone H1 was described as an integral component of the cytoskeleton in sea urchin eggs [23]. Moreover, calf histones may increase processing and presentation of antigenic peptide-major histocompatibility complexes to T cells in macrophages [24]. Therefore, the amount of histone H2A used as transfection reagent should be carefully regarded. The cell death effect of HPhA (a recombinant histone-like protein from *Pyrococcus horikoshii OT3*) was observed only at very high concentration ($>$ 100 μg/ml) and the toxicity of 1000 μg/ml HPhA was similar to that of 20 μg/ml for NIH 3T3 cells and about 40 μg/ml for HL-7702 cells transfected with LipofectamineTM, respectively [10]. Here we tested different amounts of histone H2A (5.5, 11.0 and 16.5 μg/ml of histone H2A) as transfection reagent in our experiments. Using 5.5 or 11.0 μg/ml of histone H2A as transfection reagents showed quite lower transfection efficiency compared to 16.5 μg/ml of histone H2A, which resulted in efficient dsRNA-silencing effect without any toxicity, shown by the similar cell viability between histone H2A transfected cells and untreated cells.

Cytotoxicity by transfecting cells in the presence of serum often causes cell death and serum has been reported to interfere with gene delivery for various transfection vectors both in vitro and in vivo [25–28]. Likewise transfection can be inhibited by serum proteins such as serum albumin, immunoglobulin, fibronectin, and various apolipoproteins, which bind to the surface of conventional liposomes, changing their surface charge and thereby affect their longevity, and therefore serum has to be avoided in transfection experiments in many cell systems [29]. For histones or histone-like proteins mediated transfection, this problem might be overcome since a steric barrier formed by histone/histone-like protein–DNA transfection complex inhibits the binding of serum protein on the surface of HPhA–DNA transfection complexes [10]. Thus, histone H2A could also be a good choice for some transfections when the presence of serum is necessary for the cultured cells.

In summary, compared to Effectene or liposome-based transfection methods, histone H2A-mediated dsRNA transfection resulted in a higher silencing efficiency and lower cytotoxicity. Our study demonstrates an inexpensive and non-cytotoxic dsRNA delivery system mediated by histone H2A, which may be useful for primary cell cultures of crustacean animals such as crayfish and shrimp.

Acknowledgment

This work has been supported by Formas and the Swedish Science Research Council.

References

[1] Mosammaparast N, Jackson KR, Guo Y, Brame CJ, Shabanowitz J, Hunt DF, et al. Nuclear import of histone H2A and H2B is mediated by a network of karyopherins. J Cell Biol 2001;153:251–62.
[2] Rosenbluh J, Singh SK, Gafni Y, Graessmann A, Loyter A. Non-endocytic penetration of core histones into petunia protoplasts and cultured cells: a novel mechanism for the introduction of macromolecules into plant cells. Biochim Biophys Acta 2004;1664:230–40.
[3] Zaitsev SV, Haberland A, Otto A, Vorob'ev VI, Haller H, Bottger M. H1 and HMG17 extracted from calf thymus nuclei are efficient DNA carriers in gene transfer. Gene Ther 1997;4:586–92.
[4] Balicki D, Beutler E. Histone H2A significantly enhances in vitro DNA transfection. Mol Med 1997;3:782–7.
[5] Balicki D, Reisfeld RA, Pertl U, Beutler E, Lode HN. Histone H2A-mediated transient cytokine gene delivery induces efficient antitumor responses in murine neuroblastoma. Proc Natl Acad Sci USA 2000;97:11500–4.
[6] Singh D, Rigby PW. The use of histone as a facilitator to improve the efficiency of retroviral gene transfer. Nucleic Acids Res 1996;24:3113–4.
[7] Balicki D, Putnam CD, Scaria PV, Beutler E. Structure and function correlation in histone H2A peptide-mediated gene transfer. Proc Natl Acad Sci USA 2002;99:7467–71.
[8] Deas O, Angevin E, Cherbonnier C, Senik A, Charpentier B, Levillain JP, Oosterwijk E, Hirsch F, Durrbach A. In vivo-targeted gene delivery using antibody-based nonviral vector. Hum Gene Ther 2002;13:1101–14.
[9] Esser D, Amanuma H, Yoshiki A, Kusakabe M, Rudolph R, Bohm G. A hyperthermostable bacterial histone-like protein as an efficient mediator for transfection of eukaryotic cells. Nat Biotechnol 2000;18:1211–3.
[10] Weng L, Liu D, Li Y, Cao S, Feng Y. An archaeal histone-like protein as an efficient DNA carrier in gene transfer. Biochim Biophys Acta 2004;1702:209–16.
[11] Fritz JD, Herweijer H, Zhang G, Wolff JA. Gene transfer into mammalian cells using histone-condensed plasmid DNA. Hum Gene Ther 1996;7:1395–404.
[12] Demirhan I, Hasselmayer O, Chandra A, Ehemann M, Chandra P. Histone-mediated transfer and expression of the HIV-1 tat gene in Jurkat cells. J Hum Virol 1998;1:430–40.
[13] Puebla I, Esseghir S, Mortlock A, Brown A, Crisanti A, Low W. A recombinant H1 histone-based system for efficient delivery of nucleic acids. J Biotechnol 2003;105:215–26.
[14] Söderhäll I, Bangyeekhun E, Mayo S, Söderhäll K. Hemocyte production and maturation in an invertebrate animal; proliferation and gene expression in hematopoietic stem cells of *Pacifastacus leniusculus*. Dev Comp Immunol 2003;27:661–72.
[15] Söderhäll I, Kim YA, Jiravanichpaisal P, Lee SY, Söderhäll K. An ancient role for a prokineticin domain in invertebrate hematopoiesis. J Immunol 2005;174:6153–60.
[16] May RC, Plasterk RH. RNA interference spreading in *C. elegans*. Methods Enzymol 2005;392:308–15.
[17] Feinberg EH, Hunter CP. Transport of dsRNA into cells by the transmembrane protein SID-1. Science 2003;301:1545–7.
[18] Fischle W, Wang Y, Allis CD. Histone and chromatin cross-talk. Curr Opin Cell Biol 2003;15:172–83.
[19] Lucius H, Haberland A, Zaitsev S, Dalluge R, Schneider M, Bottger M. Structure of transfection-active histone H1/DNA complexes. Mol Biol Rep 2001;28:157–65.
[20] Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell 2000;103:263–71.
[21] Brix K, Summa W, Lottspeich F, Herzog V. Extracellularly occurring histone H1 mediates the binding of thyroglobulin to the cell surface of mouse macrophages. J Clin Invest 1998;102:283–93.
[22] Class R, Lindman S, Fassbender C, Leinenbach HP, Rawer S, Emrich JG, Brady LW, Zeppezauer M. Histone H1 suppresses tumor growth of leukemia cells in vitro, ex vivo and in an animal model suggesting extracellular functions of histones. Am J Clin Oncol 1996;19:522–31.
[23] Multigner L, Gagnon J, Van Dorsselaer A, Job D. Stabilization of sea urchin flagellar microtubules by histone H1. Nature 1992;360:33–9.
[24] Dohlman JG, Pillion DJ, Rokeach LA, Ramprasad MP. Identification of macrophage cell-surface binding sites for cationized bovine serum albumin. Biochem Biophys Res Commun 1991;181:787–96.
[25] Plank C, Mechtler K, Szoka Jr. FC, Wagner E. Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous gene delivery. Hum Gene Ther 1996;7:1437–46.
[26] Gao X, Huang L. Cationic liposome-mediated gene transfer. Gene Ther 1995;2:710–22.
[27] Thierry AR, Rabinovich P, Peng B, Mahan LC, Bryant JL, Gallo RC. Characterization of liposome-mediated gene delivery: expression, stability and pharmacokinetics of plasmid DNA. Gene Ther 1997;4:226–37.
[28] Zelphati O, Uyechi LS, Barron LG, Szoka Jr. FC. Effect of serum components on the physico-chemical properties of cationic lipid/oligonucleotide complexes and on their interactions with cells. Biochim Biophys Acta 1998;1390:119–33.
[29] Audouy S, Molema G, de Leij L, Hoekstra D. Serum as a modulator of lipoplex-mediated gene transfection: dependence of amphiphile, cell type and complex stability. J Gene Med 2000;2:465–76.