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Histone H2A as a transfection agent in crayfish hematopoietic tissue cells

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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

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[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