



Title	Transgene expression efficiency from plasmid DNA delivered as a complex with histone H3
Author(s)	Kamiya, Hiroyuki; Goto, Hitomi; Kanda, Genki; Yamada, Yuma; Harashima, Hideyoshi
Citation	International Journal of Pharmaceutics, 392(1-2), 249-253 https://doi.org/10.1016/j.ijpharm.2010.03.035
Issue Date	2010-06-15
Doc URL	http://hdl.handle.net/2115/43144
Type	article (author version)
File Information	IJP392-1-2_249-253.pdf



[Instructions for use](#)

Transgene expression efficiency from plasmid DNA delivered as a complex with histone H3

Hiroyuki Kamiya^{a,b,*}, Hitomi Goto^{a,b}, Genki Kanda^{a,b}, Yuma Yamada^{a,b},
Hideyoshi Harashima^{a,b}

*^aFaculty of Pharmaceutical Sciences, Hokkaido University, Kita-12,
Nishi-6, Kita-ku, Sapporo 060-0812, Japan*

^bCREST, Japan Science and Technology, Japan

*Corresponding author. Tel: +81-11-706-3733; Fax: +81-11-706-4879.

E-mail address: hirokam@pharm.hokudai.ac.jp (H. Kamiya)

Abstract

The intranuclear disposition of plasmid DNA is extremely important for transgene expression. Exogenous histones have been used as carriers of plasmid DNA in histone-mediated gene delivery. In this study, the effects of exogenous histone H3 complexed with plasmid DNA on transgene expression efficiency were examined. The plasmid–histone complexes in various ratios were transfected into HeLa cells by osmotic pressure. Histone H3 suppressed transgene expression in the nucleus in a dose-dependent manner. Our results suggest that the histone-mediated gene delivery is unlikely to be useful, from the viewpoint of the intranuclear disposition.

Keywords: exogenous DNA, histone, H3, intranuclear disposition, expression efficiency

1. Introduction

Histones are the chief protein components of chromatin and have an important function in chromosomal gene regulation. Plasmid DNAs delivered with nonviral vectors also bind to histones, since nucleosomes are formed on non-integrated plasmid DNAs (Reeves et al., 1985). We previously proposed that the intranuclear disposition of the delivered plasmid is an important factor in efficient transgene expression (Kamiya et al., 2003). The binding of histones, in general, limits the access of transcriptional factors to their recognition sites in chromosomal DNA, and thus, the binding of histones to the exogenous DNA would also affect transgene expression. Therefore, histones could be a key factor for the intranuclear disposition of the exogenous DNA. In agreement with this hypothesis, transgene expression is affected by the introduction of DNA sequences with high and low histone affinities into plasmid DNAs (Nishikawa et al., 2003; Sumida et al., 2006; Kamiya et al., 2007, 2009).

Recently, histones (H1, H2A, H2B, H3, and H4) have been used as vehicles of plasmid DNAs (Kaouass et al., 2006 and references therein). Histones have the ability to penetrate the cell membrane, even in the presence of endocytosis inhibitors (Hariton-Gazal et al., 2003). Transfection with histone–DNA complexes is also achieved without the effect of endocytosis inhibitors (Wagstaff et al., 2007). The nuclear localizing signal (NLS) sequence of histone H2A is important for the

transfection efficiency, and the additional attachment of the NLS peptide enhances both the nuclear entry of the H2B–DNA complex and transgene expression (Wagstaff et al., 2007; Balicki et al., 2002). Thus, the histone–DNA complexes are expected to penetrate the cell membrane by a non-endocytotic pathway(s) and enter the nucleus through nuclear pores. These interesting findings, however, raise the question of whether histone-mediated gene delivery (histonefection) is advantageous, from the viewpoint of the intranuclear disposition.

In this study, we examined transgene expression efficiency from plasmid DNA complexed with histone H3, in comparison to that from plasmid DNA delivered in a naked form. For this purpose, the naked and complexed plasmid DNAs were introduced into cells by osmotic pressure, an efficient method for the introduction of molecules into cells (Koberna et al., 1999). Our results suggested that histone H3 suppressed transgene expression in a dose-dependent manner and that the histone-mediated gene delivery is unlikely to be useful, from the viewpoint of the intranuclear disposition.

2. Materials and methods

2.1. Materials

Oligodeoxyribonucleotides were purchased from Invitrogen Japan (Tokyo, Japan) in purified forms. The pYK-CMV-luc plasmid DNA (Ochiai et al., 2006) was amplified in *Escherichia coli* strain DH5 α , and was purified with a Qiagen (Hilden, Germany) EndoFree Plasmid Mega kit. The pET3a-H3.1 plasmid (Shimada et al., 2008) was kindly provided by Dr. Hideaki Tagami.

2.2. Purification of histone H3

The histone H3 protein was expressed in *E. coli* strain BL21 (DE3) containing pET3a-H3.1 by an induction with isopropyl- β -D-thiogalactopyranoside (final concentration of 0.5 mM). After lysis by sonication, the lysate was centrifuged (10,000 g, 15 min, 0°C) and the pellet (inclusion bodies) was obtained. The pellet was washed twice by suspension in 10 mM Tris-HCl (pH 8.0), sonication, and centrifugation. The pellet was suspended in 0.3 M H₂SO₄ (von Holt and Brandt, 1977), mixed by vortexing (1 min), and further mixed by agitation (110 rpm, 1 hr). After centrifugation (10,000 g, 15 min, 0°C), the supernatant was dialyzed twice against Hepes-buffered saline at 4°C. The purified H3 protein was obtained as the supernatant after centrifugation (10,000 g, 15 min, 0°C).

2.3. Transfection

HeLa cells were seeded at a density of 1×10^5 cells/well, and were incubated in Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum under a 5% CO₂ / air atmosphere at 37°C for 24 hr. The luciferase plasmid DNA (2 μg) was mixed with H3 at weight ratios of 1:0.3, 1:1, 1:3, and 1:6 (as plasmid:H3) and then incubated at room temperature for 30 min. KHB buffer (30 mM KCl, 10 mM Hepes-KOH, pH 7.4) was added to a final volume of 600 μL (Koberna et al., 1999). The medium was removed, and the cells were washed with the medium without the serum and then with KHB buffer. The solution containing the DNA-histone complex was added to the cells. After an incubation for 15 min at room temperature, the medium was changed and the cells were incubated under a 5% CO₂ atmosphere at 37°C for 24 hr. The cells were washed with phosphate-buffered saline and were released by trypsinization. After centrifugation at 13,000 g for 2 min at 4°C, the pellet was washed with PBS. The luciferase activity and the amount of exogenous DNA were measured, as described below.

2.4. Luciferase activity

The cell pellet was resuspended in Reporter Lysis Buffer (Promega, Madison, Wisconsin, USA) and was frozen and thawed. After centrifugation at 13,000 g for 2 min at 4°C, the supernatant was examined for luciferase activity, using the Luciferase Assay Systems kit (Promega).

An aliquot of the supernatant was mixed with the Luciferase Assay Reagent, and the luminescence was measured with a Luminescencer PSN (Atto, Tokyo, Japan).

2.5. Isolation of nuclear DNA and quantitative PCR

The cell pellet was resuspended in DNA lysis buffer (10 mM Tris-HCl, 10 mM NaCl, 3 mM MgCl₂, 0.5% (w/v) IGEPAL-CA630, pH 7.4) (Tachibana et al., 2002). After centrifugation at 1,400 g for 5 min at 25°C, the pellet was washed three times with DNA lysis buffer and stored at -80°C. The intranuclear DNA was extracted with the SepaGene reagent (Sanko Jun-yaku, Tokyo, Japan).

Quantitative PCR (Q-PCR) was performed using an ABI 7500 real time PCR system (Applied Biosystems, Foster City, California, USA) and SYBR-Green chemistry, as described previously (Ochiai et al., 2006), using the following primers: Luc (+), 5'-GGTCCTATGATTATGTCCGGTTATG; Luc (-), 5'-ATGTAGCCATCCATCCTTGTC AAT.

2.6. Confocal laser microscopy

The pYK-CMV-luc plasmid was labeled with Alexa Fluor 488 by the ULYSIS Nucleic Acid Labeling Kit (Molecular Probes, Eugene, OR, USA) and was purified by gel filtration. Histone H3 was labeled by Texas

Red-X succinimidyl ester (Molecular Probes) and was purified by ultrafiltration with Amicon Ultra-0.5 (nominal molecular weight 3,000) (Millipore, Billerica, Massachusetts, USA). At 3 hr after the transfection initiation, the medium was replaced by fresh medium containing Hoechst 33342 (final concentration, 5 $\mu\text{g}/\text{mL}$), and the cells were incubated for 10 min to stain the nuclei. After incubation, the cells were washed with the phenol red-free medium containing serum, and then observed by confocal laser scanning microscopy (Nikon A1; Nikon, Tokyo, Japan).

2.7. Characterization of complexes

The particle size was measured by a dynamic light scattering method and ζ -potential measurements were performed using electrophoretic light scattering method, with Zetasizer Nano-ZS (Malvern Instruments, Herrenberg, Germany).

2.8. Statistical analysis

Statistical significance was examined by the Student's *t*-test. Levels of $P < 0.05$ were considered to be significant.

3. Results

3.1. Effects of histone H3 on transgene expression from plasmids in cultured cells

We introduced plasmid DNA containing the luciferase gene by means of osmotic pressure, a method that enables delivery into the cytoplasm of living cells (Koberna et al., 1999). We used this method to compare plasmid DNAs in both the naked and complexed forms, although the histone–DNA complex could penetrate the cell membrane (Wagstaff et al., 2007). The luciferase plasmid DNA was mixed with H3 at weight ratios of 1:0.3, 1:1, 1:3, and 1:6 (as plasmid:H3), and these complexes were introduced into HeLa cells. The luciferase activities and the amounts of exogenous DNA were examined after 24 hr.

As shown in Fig. 1, the luciferase activities were similar for the naked plasmid (1:0) and the plasmid DNAs complexed with H3 at weight ratios of 1:0.3 and 1:1. Since no luciferase activity was observed for the naked plasmid without the osmotic pressure (data not shown), this method successfully delivered the naked DNA into the nucleus. The luciferase activity seemed to decrease for the plasmid-histone complexes in a dose-dependent manner. The activities for the 1:3 and 1:6 complexes were 12- and 450-fold, respectively, less than that for the naked plasmid.

No evident toxicities were observed by the complexation of the plasmid DNA with histone H3 (data not shown).

3.2. Effects of histone H3 on the amounts of exogenous DNA

Next, we determined the amounts of the exogenous DNAs in the nuclei. The complexation with histone H3 increased the amounts of intranuclear luciferase DNAs (Fig. 2). The exogenous DNAs were present in the nuclei at 18-, 52-, and 210-fold more abundance for the 1:0.3, 1:1, and 1:3 DNA–histone complexes, respectively, as compared to that for the naked plasmid. However, only a 31-fold increased amount of the luciferase DNA was observed in the case of the 1:6 complex. Although the detected amounts of the exogenous DNAs for the DNA–histone complexes were at least one order of magnitude more than that of the naked plasmid, the luciferase activities exhibited different trends, as shown in Fig. 1.

3.3. Effects of histone H3 on expression efficiency

We then calculated the expression efficiencies. The luciferase activities were divided by the amounts of the exogenous DNAs, and these values were considered to indicate the amounts of the luciferase protein expressed from one copy of the transgene. As shown in Fig. 3, the expression efficiencies clearly decreased when the amount of histone H3 increased. The efficiencies of the 1:0.3 and 1:1 complexes that produced luciferase at similar levels to the naked plasmid were 14- and 44-fold, respectively, less than that of the naked DNA. The efficiency was dramatically (2,800-fold) suppressed when the plasmid DNA was

complexed with H3 at a weight ratio of 1:3. The suppression was more evident for the 1:6 complex, and the efficiency was 18,000-fold less than that of the naked plasmid.

3.4. Confocal microscopic observation

We next introduced complexes of Alexa Fluor 488-labeled plasmid DNA and Texas Red-labeled histone H3 into HeLa cells. After 3 hr after the transfection initiation, the treated cells were analyzed by confocal laser scanning microscopy. More plasmid DNA was detected for the 1:3 and 1:6 complexes than for the 1:0.3 and 1:1 complexes under the conditions that we used (Fig. 4). This result suggests that amount of the labeled plasmid DNA was more abundant for the 1:6 complex than for the 1:1 complex, in contrast to the result obtained with the unlabeled plasmid DNA and H3 (Fig. 2). As expected, more H3 was observed when the H3 ratio to the plasmid DNA was increased. Although it was difficult to detect small-sized molecules by the microscopy, the plasmid DNA and H3 seemed to colocalize in the cases of the 1:3 and 1:6 complexes. Taken together with the results shown in Fig. 2, the complexation of plasmid DNA with H3 could deliver the DNA into the nucleus.

3.5. Characterization of plasmid DNA–histone H3 complexes

In order to better understand the reason(s) for less efficient expression efficiency by the complexation, the sizes and ζ -potentials of the complexes were measured. The DNA–H3 complexes were $\sim 1,000$ nm in size and no marked difference was observed (Table 1). The 1:0.3, 1:1, and 1:3 complexes were negatively charged while the 1:6 complex was positively charged. These properties of the complexes, however, could not explain the results shown in Fig. 3.

We finally analyzed dissociation of the complexes by agarose gel electrophoresis. Complexed and dissociated plasmid DNAs can be distinguished since only dissociated DNAs are stained by ethidium bromide. The bands corresponding to “naked” plasmid DNA decreased depending on amount of histone H3 (lanes 2–5, Fig. 5). Thus, as expected, tighter complexes formed when increased amount of H3 was mixed with plasmid DNA. This result suggests that dissociation efficiencies of the complexes with higher amount of H3 are low in cells and this could explain the suppressed expression efficiencies from the complexed plasmid DNAs.

4. Discussion

The objective of this study was to examine the influence of histone H3, which is used for histone-mediated gene delivery, on transgene expression efficiency. The results obtained in this study indicate that the complexation with histone H3 suppressed the transgene expression in the

nucleus (Fig. 3). When histone H3 (molecular weight 15.4×10^3) was mixed with plasmid DNA at a weight ratio of 1:1, the H3 was calculated to bind the DNA with one H3 molecule per 25 bp. In the case of chromosomal DNA, the nucleosome core particle consists of ~ 147 bp of DNA wrapped around a histone octamer, containing two molecules of H3, and ~ 200 bp of DNA is included in the nucleosome, when the linker DNA is also considered. Thus, the molar ratios of H3 to plasmid DNA might be quite high, as compared to the situation of chromosomal DNA. In our preliminary experiments, transgene expression by histone-mediated gene delivery was detectable for the 1:6 plasmid DNA–H3 complex, but not for the complexes with lower histone ratios (data not shown). However, the transgene expression was severely suppressed in the case of the 1:6 complex (Fig. 3). Thus, the histone-mediated gene delivery is unlikely to be useful, from the viewpoint of expression efficiency.

The importance of dissociation of plasmid DNAs and cationic compounds was previously shown. Schaffer et al. (2000) compared the effects of the length of polylysine peptides on transgene expression in cultured cells, in addition to *in vitro* transcription. They concluded that the dissociation in the nucleus was one of the barriers to efficient expression. In addition, Pollard et al. (1998) microinjected plasmid DNAs complexed with cationic lipids into the nucleus, and reported that the complexation prevented transgene expression in the nucleus. These results indicate the

importance of dissociation for efficient transgene expression in the nucleus. Thus, the dose-dependent decrease in the expression efficiency, shown in Fig. 3, would be caused by the inefficient dissociation of histone H3 and plasmid DNA in cells. In agreement with this interpretation, less efficient dissociation of plasmid DNA from the complexes with H3 was observed by agarose gel electrophoresis (Fig. 5). Quite high amounts of H3 bound to the plasmid DNA would inhibit the access of transcriptional factors to the regulatory regions on the plasmid.

The amounts of the exogenous DNAs in the nuclei were higher for the plasmid–H3 complexes than that for the naked plasmid (Fig. 2). If we hypothesize that the amounts of plasmid DNAs that entered the cytoplasm by the osmotic pressure method were similar and that the pathway of the entry was identical, then this result may suggest that the complexation was advantageous for the delivery into the nucleus. Indeed, labeled plasmid DNA in the nucleus was detected for the 1:3 and 1:6 complexes (Fig. 4). Histones have the NLS sequence, and importance of the NLS sequence was reported for histone H2A (Wagstaff et al., 2007; Balicki et al., 2002), suggesting nuclear entry through the nuclear pores. However, this interpretation should be reviewed carefully, since the DNA–H3 complexes were ~1,000 nm in size (Table 1). The 1:6 complex seemed to deliver a lower amount of plasmid DNA than the 1:3 complex ($P=0.07$). This inversion phenomenon might be due to partial inhibition of the nuclear

entry function of the NLS sequence by the presence of too large of an amount of H3 in the 1:6 complex. Alternatively, the effects of the complexation with H3 on the amounts of the exogenous DNAs in the nucleus may be explained by the protection of the plasmid DNA from attacks by nucleases. However, as described above, the complexation was disadvantageous, in terms of the expression efficiency.

Efficient transgene expression is required in gene therapy. The intranuclear disposition of the delivered plasmid is an important factor in efficient transgene expression (Kamiya et al., 2003). Nucleosomes are formed on non-integrated plasmid DNAs (Reeves et al., 1985), and the introduction of high and low histone-affinity DNA sequences into plasmid DNAs influences transgene expression (Nishikawa et al., 2003; Sumida et al., 2006; Kamiya et al., 2007, 2009). Moreover, decreased expression efficiency (silencing) seems to be accompanied by increased histone binding, not by histone modification (Ochiai et al., 2007). Therefore, the control of the interaction(s) between the plasmid DNA and the histones seems to be one of the keys for controlling the intranuclear disposition. The histone-mediated delivery of plasmid DNAs requires excess amounts of histones, which suppress efficient transgene expression. Thus, simply mixing plasmid DNAs and histones limits the usefulness of histones as DNA carriers. Recently, Jans and co-workers reported transfection with reconstituted chromatin (chromofection) (Wagstaff et al., 2008). They

mixed the histone octamer and the plasmid at a molar ratio of 30 octamers/plasmid, under the assumption that every octamer binds to 208 bp DNA. If the histone octamers are actually distributed homogeneously (~one octamer/208 bp), then the transgene could be expressed without severe suppression by an excess amount of histones.

In conclusion, the complexation with histone H3 decreased the transgene expression from plasmid DNA in cells. Since histones seem to be one of the keys for controlling the intranuclear disposition, properly controlled interaction(s) of plasmid DNA with histones are important. The use of exogenous histones and functional DNA sequences that affect the interaction(s) are some of the ways to control the plasmid–histone interaction. Further studies are necessary with regard to these possibilities.

Acknowledgments

We thank Dr. Hideaki Tagami (Nagoya City University) for the pET3a-H3.1 plasmid. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and from the Japan Society for the Promotion of Science.

References

- Balicki, D., Putnam, C.D., Scaria, P.V., Beutler, E., 2002. Structure and function correlation in histone H2A peptide-mediated gene transfer. *Proc. Natl. Acad. Sci. U.S.A.*, 99, 7467-7471.
- Hariton-Gazal, E., Rosenbluh, J., Graessmann, A., Gilon, C., Loyter, A., 2003. Direct translocation of histone molecules across cell membranes. *J. Cell Sci.*, 116, 4577-4586.
- Kamiya, H., Akita, H., Harashima, H., 2003. Pharmacokinetic and pharmacodynamic considerations in gene therapy. *Drug Discov. Today*, 8, 960-966.
- Kamiya, H., Fukunaga, S., Ohyama, T., Harashima, H., 2007. The location of the left-handedly curved DNA sequence affects exogenous DNA expression *in vivo*. *Arch. Biochem. Biophys.*, 461, 7-12.
- Kamiya, H., Goto, H., Harashima, H., 2009. Effects of non-B DNA sequences on transgene expression. *J. Biosci. Bioengng.*, 108, 20-23.
- Kaouass, M., Beaulieu, R., Balicki, D., 2006. Histonefection: Novel and potent non-viral gene delivery. *J. Control. Release*, 113, 245-254.
- Koberna, K., Stanev^ˇk, D., Malínský, J., Eltsov, M., Pliss, A., C^ˇtrnáctá, V., Cermanová, S^ˇ., Ras^ˇka, I., 1999. Nuclear organization studied with the help of a hypotonic shift: its use permits hydrophilic molecules to enter into living cells. *Chromosoma*, 108, 325-335.

- Nishikawa, J., Amano, M., Fukue, Y., Tanaka, S., Kishi, H., Hirota, Y., Yoda, K., Ohyama, T., 2003. Left-handedly curved DNA regulates accessibility to cis-DNA elements in chromatin. *Nucleic Acids Res.*, 31, 6651-6662.
- Ochiai, H., Harashima, H., Kamiya, H., 2006. Intranuclear disposition of exogenous DNA *in vivo*: silencing, methylation and fragmentation. *FEBS Lett.*, 580, 918-922.
- Ochiai, H., Fujimuro, M., Yokosawa, H., Harashima, H., Kamiya, H., 2007. Transient activation of transgene expression by hydrodynamics-based injection may cause rapid decrease in plasmid DNA expression. *Gene Ther.*, 14, 1152-1159.
- Pollard, H., Remy, J.S., Loussouarn, G., Demolombe, S., Behr, J.P., Escande, D., 1998. Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus in mammalian cells. *J. Biol. Chem.*, 273, 7507-7511.
- Reeves, R., Gorman, C.M., Howard, B., 1985. Minichromosome assembly of non-integrated plasmid DNA transfected into mammalian cells. *Nucleic Acids Res.*, 13, 3599-3615.
- Schaffer, D.V., Fidelman, N.A., Dan, N., Lauffenburger, D.A., 2000. Vector unpacking as a potential barrier for receptor-mediated polyplex gene delivery. *Biotechnol. Bioeng.*, 67, 598-606.

- Shimada, M., Niida, H., Zineldeen, D.H., Tagami, H., Tanaka, M., Saito, H., Nakanishi, M., 2008. Chk1 is a histone H3 threonine 11 kinase that regulates DNA damage-induced transcriptional repression. *Cell*, 132, 221-232.
- Sumida, N., Nishikawa, J., Kishi, H., Amano, M., Furuya, T., Sonobe, H., Ohyama, T., 2006. A designed curved DNA segment that is a remarkable activator of eukaryotic transcription. *FEBS J.*, 273, 5691-5702.
- Tachibana, R., Harashima, H., Ide, N., Ukistu, S., Ohta, Y., Suzuki, N., Kikuchi, H., Shinohara, Y., Kiwada, H., 2002. Quantitative analysis of correlation between number of nuclear plasmids and gene expression activity after transfection with cationic liposomes. *Pharm. Res.*, 19, 377-381.
- von Holt, C., Brandt, W.F., 1977. Fractionation of histones on molecular sieve matrices. *Methods Cell Biol.*, 16, 205-225.
- Wagstaff, K.M., Glover, D.J., Tremethick, D.J., Jans, D.A., 2007. Histone-mediated transduction as an efficient means for gene delivery. *Mol. Ther.*, 15, 721-731.
- Wagstaff, K.M., Fan, J.Y., De Jesus, M.A., Tremethick, D.J., Jans, D.A., 2008. Efficient gene delivery using reconstituted chromatin enhanced for nuclear targeting. *FASEB J.*, 22, 2232-2242.

FIGURE LEGENDS

Figure 1. Expression of the luciferase gene from plasmid DNAs complexed with histone H3. Plasmid DNA (2 μg) was mixed with recombinant H3 at weight ratios of 1:0 (naked plasmid), 1:0.3, 1:1, 1:3, and 1:6. The complexes were introduced into HeLa cells by osmotic pressure, as described in the Materials and Methods section. The luciferase activities were measured after 24 hr. The values represent the averages of three separate experiments. Bars represent SD. * $P < 0.05$ and ** $P < 0.01$ versus 1:0 (naked plasmid).

Figure 2. Amounts of luciferase DNA in the nucleus. Plasmid DNA–H3 complexes were introduced into HeLa cells by osmotic pressure, as described in the Materials and Methods section. The amounts of the exogenous DNA were measured after 24 hr. The values represent the averages of three separate experiments. Bars represent SD. * $P < 0.05$ and *** $P < 0.001$ versus 1:0 (naked plasmid).

Figure 3. Expression efficiencies of the luciferase gene from plasmid DNAs complexed with histone H3. Plasmid DNA–H3 complexes were introduced into HeLa cells by osmotic pressure, as described in the Materials and Methods section. The luciferase activities, shown in Fig. 1,

were divided by the amounts of the exogenous DNAs, shown in Fig. 2. The values represent the averages of three separate experiments. Bars represent SD. * $P < 0.05$ and ** $P < 0.01$ versus 1:0 (naked plasmid).

Figure 4. Confocal microscopic observation. Alexa Fluor 488-labeled plasmid DNA and Texas Red-labeled histone H3 ((A) 1:0.3, (B) 1:1, (C) 1:3, and (D) 1:6, weight ratio) complexes were introduced into HeLa cells. After 3 hr after the transfection initiation, nuclei were stained with Hoechst 33342 prior to observation by confocal laser scanning microscopy. The cells were excited by 405 nm light from Diode laser, 488 nm light from an Ar laser and 561 nm light from a DPSS laser. A series of images were obtained using a Nikon A1 with a water immersion objective lens (Plan Apo 60x1.20 PFS WI) and a 1st dichroic mirror (405/488/561/640). The three fluorescence detection channels were set to the following filters: 450/50 (blue) for nucleus, 525/50 (green) for plasmid DNA and 595/50 (red) for histone H3. Note that plasmid DNA was observed as a yellow cluster when it was colocalized with histone H3. Scale bars, 10 μm . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Figure 5. Dissociation of plasmid DNA–histone H3 complexes. Plasmid DNA (2 μg) was mixed with recombinant H3 at weight ratios of 1:0 (naked

plasmid), 1:0.3, 1:1, 1:3, and 1:6. The complexes containing 100 ng DNA (10 μ L in HEPES-buffered saline) were mixed with 2 μ L of gel-loading solution (40% sucrose, 1 mM EDTA, 0.25% bromophenol blue) and applied to a 1% agarose gel. The gel was stained with ethidium bromide. Lane M, marker DNAs (λ DNA/Sty I); lane 1, naked plasmid; lane 2, 1:0.3 complex; lane 3, 1:1 complex; lane 4, 1:3 complex; lane 5, 1:6 complex.

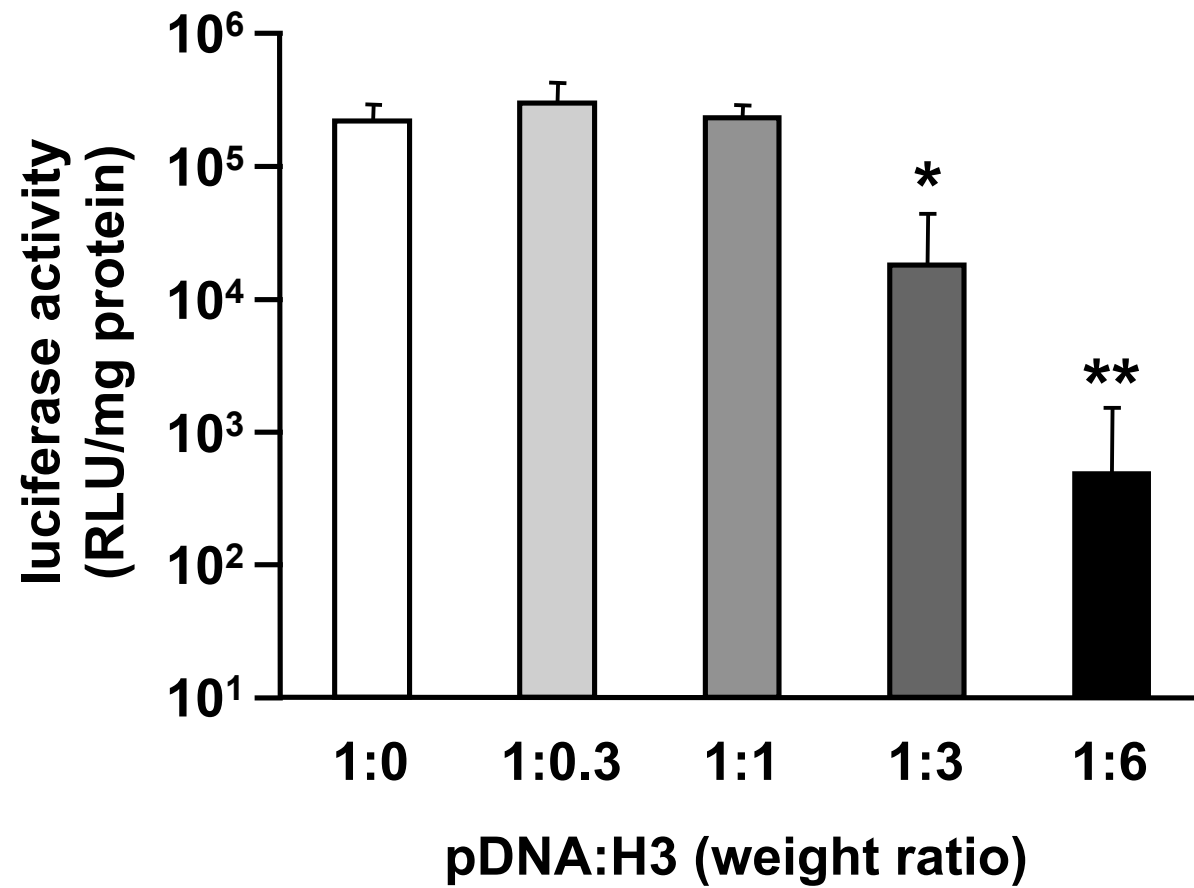


Fig. 1

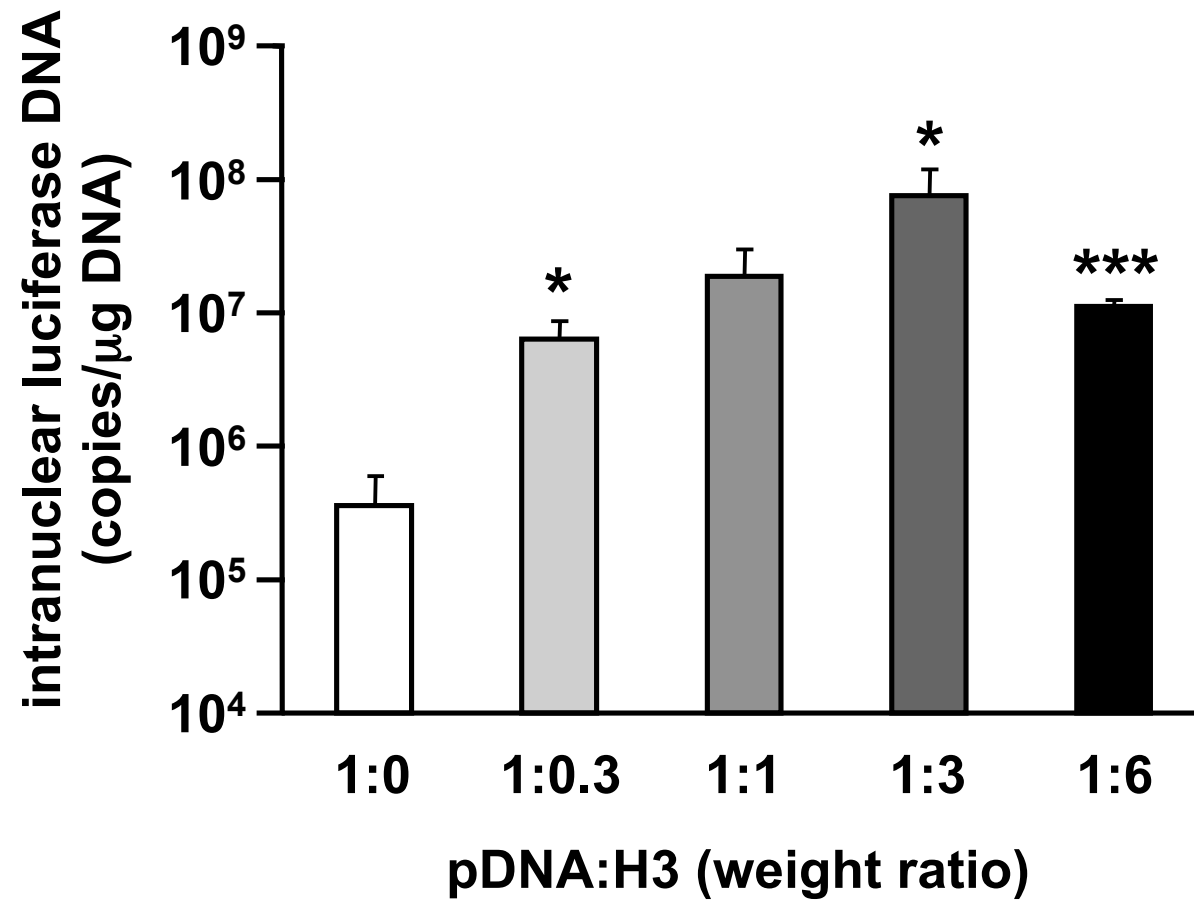


Fig. 2

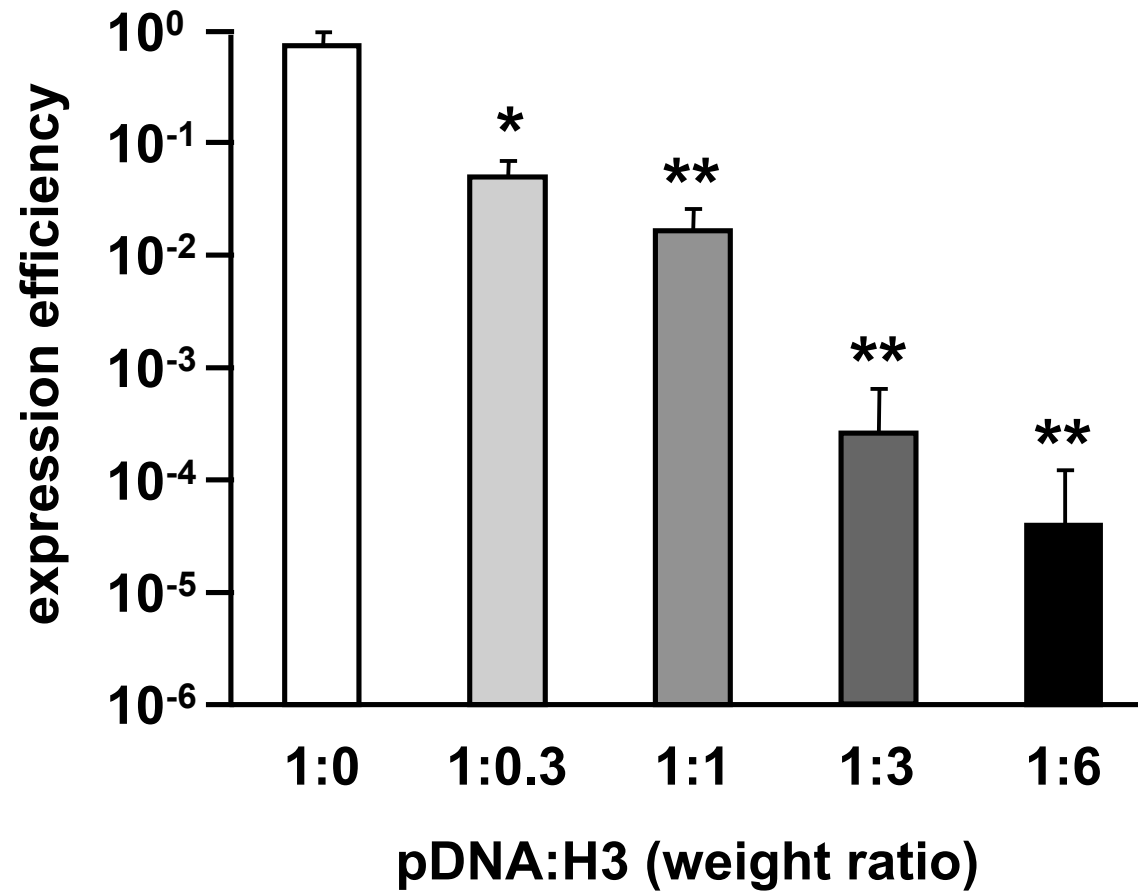


Fig. 3

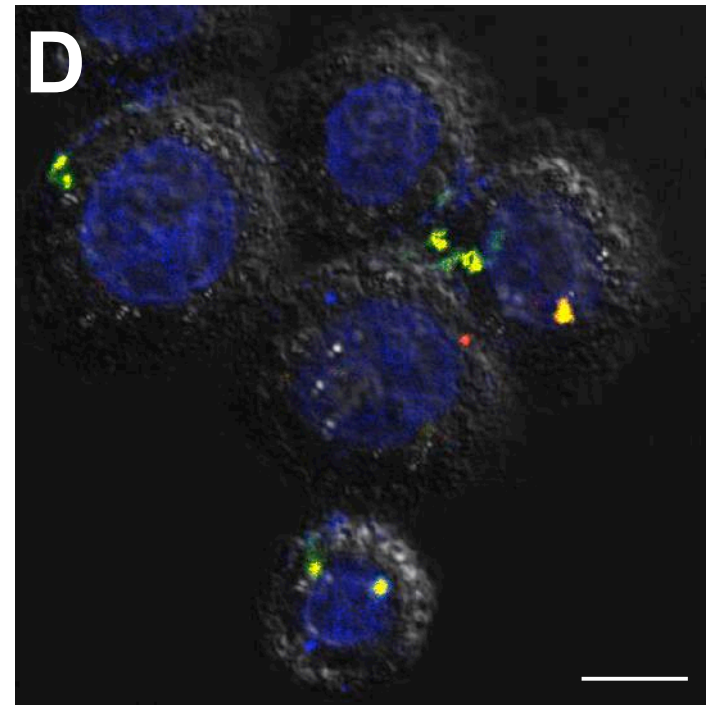
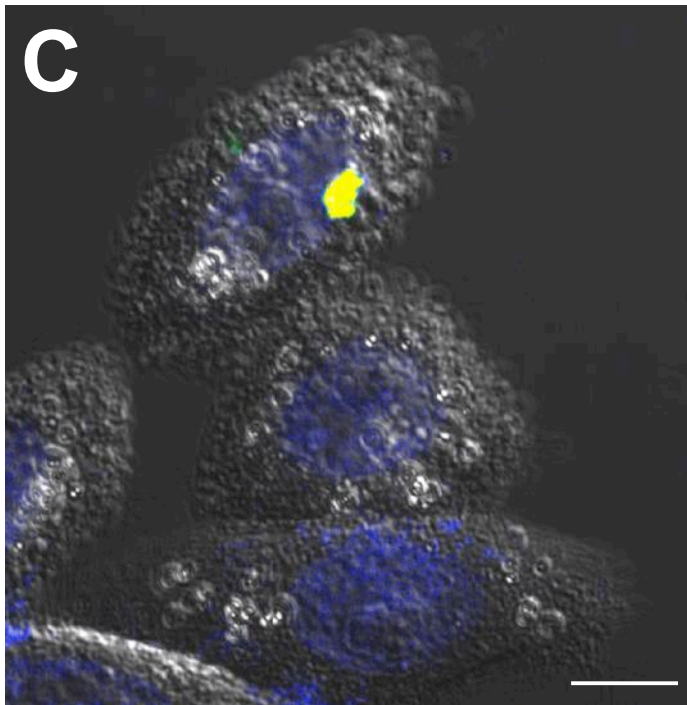
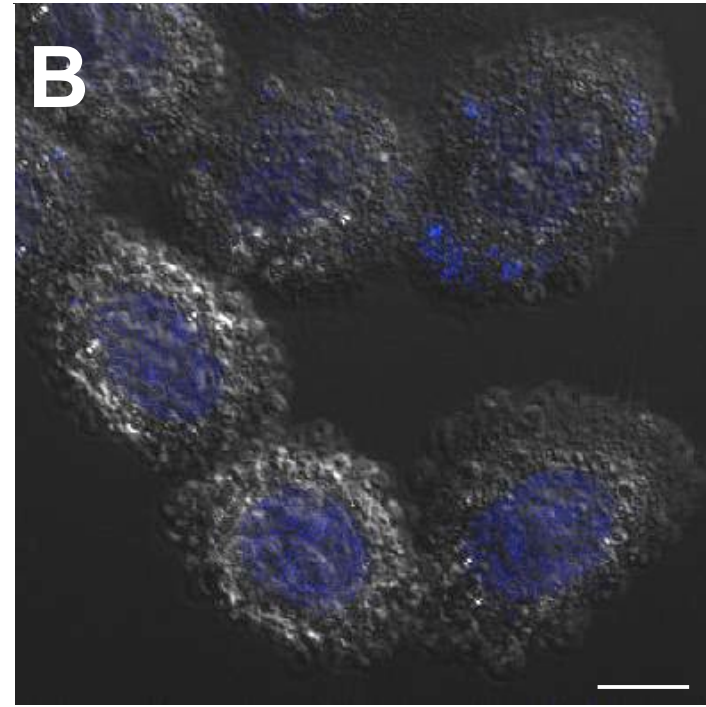
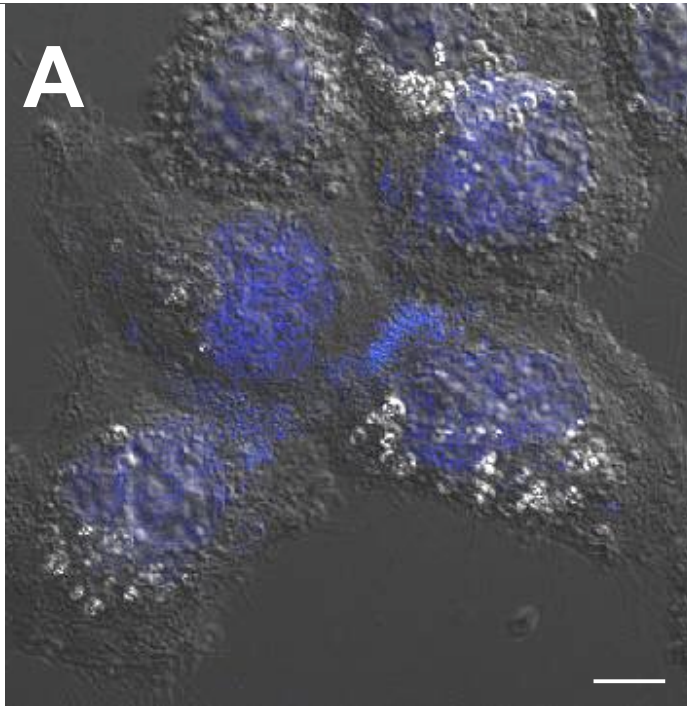


Fig. 4

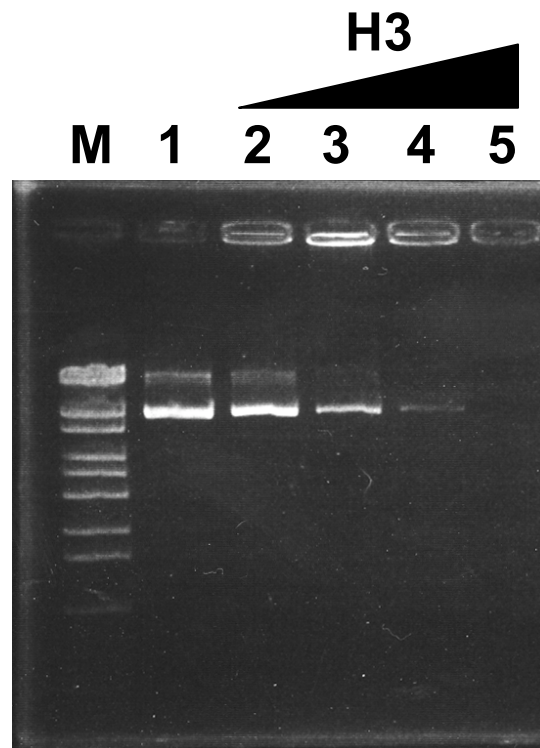


Fig. 5

Table 1. Characterization of plasmid DNA and histone H3 complexes

pDNA:H3 (weight ratio)	1:0.3	1:1	1:3	1:6
diameter (nm)	1109	1178	877	1167
ζ -potential (mV)	-19	-27	-23	13

Experiments were done in duplicate and the mean values are represented.