Effects of carriers on transgene expression from plasmids containing a DNA sequence with high histone affinity

Hiroyuki Kamiya\textsuperscript{a,b,*}, Satoki Fukunaga\textsuperscript{a,b}, Takashi Ohyama\textsuperscript{c}, and Hideyoshi Harashima\textsuperscript{a,b}

\textsuperscript{a} Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan
\textsuperscript{b} CREST, Japan Science and Technology, Japan
\textsuperscript{c} Department of Biology, Faculty of Education and Integrated Arts and Sciences, Waseda University, 2-2, Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, 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 highly important for transgene expression. The effects of a left-handedly curved sequence with high histone affinity on transgene expression were examined in COS-7 cells with two kinds of carriers (Lipofectamine Plus and TransIT-LT1). Three plasmids containing the curved sequence at different positions were transfected. The transgene expression was affected by the position of the left-handedly curved sequence, and the sequence at appropriate locations enhanced the expression from plasmid DNAs. However, the position effects on the expression differed from those obtained by electroporation of the same plasmid DNAs in a naked form. In addition, the degree of expression enhancement seemed to depend on the carriers. These results suggest that the left-handedly curved sequence with high histone affinity could increase the transgene expression from a plasmid delivered with carriers.

Keywords: left-handedly curved DNA; histones; plasmid DNA; intranuclear disposition; carriers
1. Introduction

Plasmid DNAs, in either the naked or complexed form, have been used for transgene expression with nonviral vectors in gene therapy and biotechnology (Mahato et al., 1997; Rolland, 1998; Kamiya et al., 2001, 2003; Niidome and Huang, 2002). The intranuclear disposition of the plasmid DNA is a key factor in efficient transgene expression (Kamiya et al., 2003). Improvements in both the intranuclear disposition of the plasmid DNA and intracellular trafficking are required, for overcoming the low transgene expression efficiency of nonviral vectors in comparison to that of viral vectors. In addition, transgene expression was found to be transient due to a decrease in the transcription efficiency (Herweijer et al., 2001; Chen et al., 2003, 2004; Pringle et al., 2005; Ochiai et al., 2006a, 2006b, 2007), although prolonged transgene expression was described in some cases (Stoll et al., 2001, Yew et al., 2002; Aliño et al., 2003; Chen et al., 2003, 2004; Hodges et al., 2004). Thus, the intranuclear disposition of the plasmid DNA is also a key factor for sustained transgene expression.

We have hypothesized that histone proteins, which have an important function in chromosomal gene regulation, are one of the key factors controlling the intranuclear disposition. Nucleosomes are reportedly formed on non-integrated plasmid DNAs (Reeves et al., 1985). The binding mode of histones to the plasmid DNA could affect transgene expression.
Thus, the interaction(s) between the plasmid DNA and the histone proteins seem to be quite important for efficient transgene expression.

Previously, Nishikawa et al. (2003) reported that TCAGTTTTT[CATGTTTTT]₃ (left-handedly curved DNA, T4), with the appropriate combination of distance and spatial positioning, could activate a eukaryotic promoter when delivered in a naked form into cultured COS-7 cells. This finding prompted us to examine the effects of this left-handedly curved sequence on transgene expression from plasmid DNAs delivered with carriers in COS-7 cells. In this study, we investigated the effects of this sequence on plasmid DNAs delivered by two commercially available transfection reagents, Lipofectamine Plus (referred to as Lipofectamine, hereafter) and TransIT-LT1, as models. We found that plasmid DNAs containing the T4 sequence at appropriate positions also activated transgene expression, when introduced in a complexed form. Interestingly, however, the effects of the distance between the T4 sequence and the TATA box (the core promoter sequence) were different from those when the naked plasmid DNAs were delivered. In addition, the degree of the enhancement was dependent on the carriers used. These results indicate that the T4 sequence is an effective functional sequence to control the intranuclear disposition of plasmid DNAs, and that carriers affect the intranuclear disposition.
2. Materials and methods

2.1. Materials

Oligodeoxyribonucleotides were purchased from Sigma Genosys Japan (Ishikari, Japan) in purified forms. The pST0/TLN-7, pLHC4/TLN+47, pLHC4/TLN-16, and pLHC4/TLN-6 plasmids, containing the thymidine kinase (tk) promoter and the luciferase gene (Fig. 1) (Nishikawa et al., 2003), were amplified in Escherichia coli and purified with a Qiagen (Hilden, Germany) EndoFree Plasmid Mega kit.

2.2. Transfection

DNA transfection with the Lipofectamine Plus Reagent (Invitrogen, Carlsbad, California, USA) was performed essentially according to the supplier’s instructions. COS-7 cells were seeded at a density of 1 X 10^5 cells/well and 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. One µg of the plasmid containing the luciferase gene and 6 µl of Plus reagent were incubated in serum-free DMEM for 15 min at room temperature, mixed with the serum-free DMEM with 4 µl of Lipofectamine, and then further incubated at room temperature for 15 min. The DNA-lipid complex was transfected into the
cells. After an incubation for a total of 3 hr under a 5% CO\textsubscript{2} atmosphere at 37°C, the medium was changed and the cells were further incubated at 37°C. The medium was changed at 48 hr intervals. The cells were washed with PBS before the luciferase assay.

DNA transfection with the TransIT-LT1 Reagent (Mirus Bio, Madison, Wisconsin, USA) was also performed essentially according to the supplier’s instructions. COS-7 cells were seeded at a density of 1 × 10\textsuperscript{5} cells/well and incubated in DMEM with 10% fetal calf serum under a 5% CO\textsubscript{2} / air atmosphere at 37°C for 24 hr. One µg of the plasmid and 3 µl of the TransIT-LT1 reagent were incubated in 200 µl of serum-free DMEM for 15 min at room temperature. The complex was then added to the cells in serum-containing media. After an incubation for a total of 48 hr under a 5% CO\textsubscript{2} atmosphere at 37°C, the medium was changed and the cells were further incubated at 37°C. The medium was changed at 48 hr intervals. The cells were washed with PBS before the luciferase assay.

2.3. Luciferase activity and quantitative PCR

Luciferase activity was measured with a Luciferase Assay System with a Reporter Lysis Buffer kit (Promega, Madison, Wisconsin, USA), according to the supplier’s instructions.

The DNA was extracted with the SepaGene reagent (Sanko Jun-yaku, Tokyo, Japan). Quantitative polymerase chain reaction (Q-PCR)
was performed using an ABI 7500 real time PCR system and SYBR-Green chemistry (Applied Biosystems, Foster City, California, USA). A portion of the recovered DNA was analyzed by Q-PCR. The luciferase gene in the cells was detected using the following primers: Luc (+), 5'-dGGTCCTATGATTATGTCCGGTTATG; Luc (-), 5'-dATGTAGCCATCCATCCTTGCAAT.

2.4. 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 the left-handedly curved sequence on gene expression from plasmid DNAs delivered with Lipofectamine

First, we delivered the plasmid DNAs with Lipofectamine (with the Plus reagent). Lipofectamine is a 3:1 (w/w) liposome formulation of the polycationic lipid 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE). The transfected COS-7 cells were
harvested, and the luciferase activity and the amounts of exogenous DNA were measured.

At 24 hr after transfection initiation, the luciferase expression from pLHC4/TLN-16 and pLHC4/TLN-6 seemed to be higher than that from pST0/TLN-7, the control plasmid, and the difference between pLHC4/TLN-16 and pST0/TLN-7 was statistically significant (Fig. 2A). At 48-96 hr, the luciferase activity was significantly higher for pLHC4/TLN-16 and pLHC4/TLN-6 than for pST0/TLN-7 (Fig. 2B-D). On the other hand, the expression from pLHC4/TLN+47 seemed lower than that from the control plasmid at 48 and 96 hr, and significantly lower at 72 hr. The expression from pLHC4/TLN-16 and pLHC4/TLN-6 was similar at 48-96 hr, in contrast to the previous observation obtained by electroporation into the same cell line (Nishikawa et al., 2003). The luciferase expression from these plasmid DNAs was nearly 4-fold higher than that from pST0/TLN-7 at 48-96 hr.

We then quantitated the amounts of the exogenous DNAs by Q-PCR (Table 1, upper panel). The amounts of the DNAs were broadly equal at each time point, and no tendency was observed for the amounts of the DNAs. These results suggest that the location of the T4 sequence did not affect the amounts of the plasmid DNAs.
3.2. Effects of the left-handedly curved sequence on gene expression from plasmid DNAs delivered with TransIT-LT1

Next, we delivered the plasmid DNAs with TransIT-LT1, a protein/polyamine-based reagent that contains histone and a unique lipid, and examined the luciferase activities and the amounts of exogenous DNA.

As shown in Fig. 3, the luciferase activity was significantly higher for pLHC4/TLN-16 and pLHC4/TLN-6 than for pST0/TLN-7, except for pLHC4/TLN-6 at 24 hr. On the other hand, the expression from pLHC4/TLN+47 seemed lower than that from the control plasmid at 24, 72 and 96 hr, and significantly lower at 48 hr. As in the case of Lipofectamine, and in contrast to the case of electroporation, the transgene expression from pLHC4/TLN-16 and pLHC4/TLN-6 was similar. The enhancement in the luciferase expression from these plasmid DNAs was 5.5- to 7.7-fold larger than that from pST0/TLN-7 at 24, 48, and 96 hr, and 14.9- and 13.7-fold (for pLHC4/TLN-16 and pLHC4/TLN-6, respectively) at 72 hr. The effects of the T4 sequence were more evident with the TransIT-LT1 reagent than with Lipofectamine (Figs. 2 and 3).

The amounts of the DNAs were broadly equal at each time point, and no tendency was observed for the amounts of the DNAs (Table 1, lower panel). These results suggest that the location of the T4 sequence did not affect the amounts of plasmids, as in the case of Lipofectamine.
3.3. Expression efficiency

We calculated the expression efficiencies for the plasmid DNAs used in this study. The luciferase activities at 24-96 hr were divided by the amounts of the exogenous DNAs at the same time points. As expected, the expression efficiencies of pLHC4/TLN-16 and pLHC4/TLN-6 were higher than those of pST0/TLN-7 and pLHC4/TLN+47 (Table 2).

Interestingly, at 48 hr, the expression efficiency from pLHC4/TLN-16 was 2-fold higher with Lipofectamine than with TransIT-LT1 (statistically significant, $P<0.05$). In contrast, the efficiency was similar in the case of pLHC4/TLN-6 at the same time point. In addition, the expression efficiencies from pLHC4/TLN-6 and pLHC4/TLN-16 relative to that from pST0/TLN-7 were larger in the case of TransIT-LT1, as compared to Lipofectamine, at 72 hr. The TransIT-LT1 reagent contains histone (although the details are proprietary) and this may be related to this observation.

4. Discussion

Previously, Nishikawa et al. (2003) introduced various plasmid DNAs, containing the left-handedly curved sequence (T4, TCAGTTTTT[CATGTTTTT]$_3$) with high histone affinity in the upstream region of the $tk$ promoter, into simian COS-7 cells by electroporation (in a naked form) and compared the luciferase expression from these plasmid
DNAs. The luciferase expression was dependent on the distance and the spatial positioning of the T4 sequence, and was enhanced when the sequence was located at appropriate positions. The pLHC4/TLN-6 and pLHC4/TLN-16 plasmids were the best and second best ones, and the expression from the pLHC4/TLN+47 plasmid was the least efficient among the plasmids containing the T4 sequence. The difference in the transgene expression efficiency could be attributed to the exposure frequency of the TATA box in the linker region, as a consequence of nucleosome formation at the T4 sequence (Nishikawa et al., 2003).

These results raised the question of whether the effects of the T4 sequence could be observed when these plasmid DNAs are delivered by carriers. In this study, we transfected the pLHC4/TLN-6, pLHC4/TLN-16, and pLHC4/TLN+47 plasmids into COS-7 cells. We also examined the pST0/TLN-7 plasmid, containing the straight sequence instead of the T4 sequence, as a control. These plasmid DNAs were introduced with two commercially available transfection reagents, Lipofectamine and TransIT-LT1, as models.

In this study, we observed that the T4 sequence also increased transgene expression in COS-7 cells in a distance-dependent manner (Figs. 2 and 3). Thus, the T4 sequence was a useful functional sequence that could control the intranuclear disposition of plasmid DNAs delivered with carriers. A comparison of the expression efficiencies per single copy of the
luciferase gene revealed that pLHC4/TLN-6 produced luciferase ~2-fold more efficiently than pLHC4/TLN-16 at 24 hr after transfection with the Lipofectamine and TransIT-LT1 reagents (Table 2). This result was similar to the observation in the previous study that the expression of the luciferase gene from the former plasmid was ~2-fold higher than that from the latter plasmid at 21 hr after electroporation (Nishikawa et al., 2003).

However, different tendencies were also observed between the previous study and this one. The luciferase gene was expressed in the order of pLHC4/TLN-6 > pLHC4/TLN-16 at 21 hr after delivery in a naked form (Nishikawa et al., 2003). On the other hand, the luciferase protein was produced from pLHC4/TLN-6 and pLHC4/TLN-16 with similar efficiencies at 48-96 hr, when transfected with the carriers used in this study (Figs. 2 and 3). Further studies are necessary to reveal the time-dependent effects of the T4 sequence on transgene expression from plasmid DNAs delivered in the naked and complexed forms.

In addition, the effects of the T4 sequence were quantitatively different with these carriers. For example, more than 10-fold higher levels of luciferase were produced from pLHC4/TLN-6 and pLHC4/TLN-16, as compared to the level of pST0/TLN-7 with TransIT-LT1 at 72 hr, and the levels was only ~3.5-fold higher with Lipofectamine at the same time point (Figs. 2 and 3). The increase in the transgene expression was larger with the former reagent than with the latter at 48-96 hr. Lipofectamine and
TransIT-LT1 seemed to affect the expression efficiencies per single copy of the transgene differently for both plasmid DNAs. In the case of pLHC4/TLN-6, the expression efficiencies were comparable for the two reagents at 48 hr (Table 2). The expression efficiencies from pLHC4/TLN-16 with Lipofectamine were higher than those with TransIT-LT1. These differences suggest that the carriers affected the intranuclear disposition of the delivered plasmid DNAs in COS-7 cells.

Recently, we administered the same plasmid DNAs in a naked form into mouse liver by a rapid, high-volume injection method (hydrodynamics-based administration), and found that the luciferase gene was expressed in the order of pLHC4/TLN-6 \( > \) pLHC4/TLN-16 \( > \) pLHC4/TLN+47 \textit{in vivo} (Kamiya et al., 2007). The same order was also observed in the liver when complexes of plasmid DNAs and the Lipofectin reagent, another nonviral vector, were administered by the hydrodynamics-based method. In this case, the carrier did not affect the order of transgene expression.

In this study, we introduced the plasmid DNAs in a complexed form. We previously proposed that the DNA-carrier complexes are converted to the DNA-histone complexes by direct replacement (Akita et al., 2007). The effects of the T4 sequence on the transgene expression from plasmid DNAs delivered with the two carriers may be derived from this replacement process. The presence of the high histone affinity T4 sequence
could accelerate the replacement, and its kinetics could be affected by affinity of cationic compounds (carriers) for DNA. In addition, it is known that the binding mode of histones for DNA has an influence on gene expression by limiting the access of transcriptional factors. Moreover, the binding mode of histones might alter stability of plasmid DNAs (note that the amount of plasmid DNA shown in Table 1 might include that of the DNA complexed with the carrier, the complex stable in cells). Thus, the high histone affinity sequence could affect various important factors for efficient transgene expression. Further studies are necessary to reveal the mechanisms of enhanced transgene expression by the T4 sequence from plasmid DNAs delivered with carriers.

In conclusion, the left-handedly curved sequence T4 enhanced the transgene expression from plasmid DNAs delivered with carriers. Thus, this sequence could be used as a functional sequence to control the intranuclear disposition of plasmid DNAs. The carriers produced different quantitative effects on this enhancement, indicating that the carriers also affect the intranuclear disposition of the plasmid DNAs. Thus, carriers should be designed by considering their effects on the intranuclear disposition of plasmid DNAs.

Acknowledgment
This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, from the Japan Society for the Promotion of Science, and from the Akiyama Foundation.
References


with enhanced safety and long-term gene expression in vivo. Mol. Ther., 5, 731-738.
FIGURE LEGENDS

**Figure 1.** Structure of the plasmid containing the left-handedly curved sequence and the *tk* promoter. T4, left-handedly curved sequence; TATA, TATA box; *tk pro*, *tk* promoter; *luc*, luciferase gene; polyA, polyA signal; *amp*, *E. coli* ampicillin resistance gene; *ori*, *E. coli* replication origin. The pST0/TLN-7 plasmid containing straight sequence is also shown.

**Figure 2.** Expression of the luciferase gene, examined by transfection of the plasmid DNAs with the Lipofectamine reagent. One µg of the plasmid DNA was transfected into COS-7 cells with Lipofectamine, and the luciferase activities were measured at (A) 24 hr, (B) 48 hr, (C) 72 hr, and (D) 96 hr. Hatched columns, pST0/TLN-7; white columns, pLHC4/TLN+47; gray columns, pLHC4/TLN-16; black columns, pLHC4/TLN-6. The values represent the averages of three independent experiments. Bars indicate SD (standard deviation). RLU, relative light units. (*P<0.05, **P<0.01, and ***P<0.001 versus pST0/TLN-7).

**Figure 3.** Expression of the luciferase gene, examined by transfection of the plasmid DNAs with the TransIT-LT1 reagent. One µg of the plasmid DNA was transfected into COS-7 cells with TransIT-LT1, and the luciferase activities were measured at (A) 24 hr, (B) 48 hr, (C) 72 hr, and
(D) 96 hr. Hatched columns, pST0/TLN-7; white columns, pLHC4/TLN+47; gray columns, pLHC4/TLN-16; black columns, pLHC4/TLN-6. The values represent the averages of three independent experiments. Bars indicate SD (standard deviation). RLU, relative light units. (*P<0.05 and **P<0.01 versus pST0/TLN-7).
Fig. 1
Fig. 2
Fig. 3
Table 1. Amounts of luciferase-DNAs in cells

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Plasmid</th>
<th>Time (hr)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>96</td>
</tr>
<tr>
<td>Lipofectamine</td>
<td>pST0/TLN-7</td>
<td></td>
<td>6.0 (±0.7) X 10^10</td>
<td>5.5 (±1.5) X 10^10</td>
<td>2.8 (±0.7) X 10^10</td>
<td>2.7 (±0.8) X 10^10</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN+47</td>
<td></td>
<td>2.9 (±0.4) X 10^10</td>
<td>3.5 (±0.6) X 10^10</td>
<td>2.6 (±0.2) X 10^10</td>
<td>2.2 (±0.2) X 10^10</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-16</td>
<td></td>
<td>2.9 X 10^10</td>
<td>4.7 (±1.7) X 10^10</td>
<td>2.5 (±0.5) X 10^10</td>
<td>1.6 (±0.0) X 10^10</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-6</td>
<td></td>
<td>4.8 (±1.0) X 10^10</td>
<td>5.6 (±2.5) X 10^10</td>
<td>3.0 (±0.3) X 10^10</td>
<td>2.6 (±0.9) X 10^10</td>
</tr>
<tr>
<td>TransIT-LT1</td>
<td>pST0/TLN-7</td>
<td></td>
<td>12 (±6) X 10^10</td>
<td>3.7 (±0.7) X 10^10</td>
<td>2.6 (±0.6) X 10^10</td>
<td>1.2 (±0.2) X 10^10</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN+47</td>
<td></td>
<td>11 (±7) X 10^10</td>
<td>11 (±9) X 10^10</td>
<td>1.8 (±0.4) X 10^10</td>
<td>1.3 (±0.1) X 10^10</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-16</td>
<td></td>
<td>15 (±11) X 10^10</td>
<td>8.9 (±1.5) X 10^10</td>
<td>2.4 (±0.9) X 10^10</td>
<td>1.5 (±0.2) X 10^10</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-6</td>
<td></td>
<td>7.2 (±4.5) X 10^10</td>
<td>5.0 (±1.6) X 10^10</td>
<td>2.3 (±1.4) X 10^10</td>
<td>1.0 (±0.2) X 10^10</td>
</tr>
</tbody>
</table>

\(^\text{a}\)Amounts of luciferase DNAs are expressed as copies/µg DNA. The values represent the averages (±SD) of three separate experiments, except for one sample (Lipofectamine, pLHC4/TLN-16, 24 hr, n=2).
Table 2. Expression efficiencies of luciferase-DNAs

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Plasmid</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipofectamine</td>
<td>pST0/TLN-7</td>
<td>0.36 (±0.22) X 10^-3</td>
<td>1.6 (±0.2) X 10^-3</td>
<td>2.5 (±0.7) X 10^-3</td>
<td>0.33 (±0.17) X 10^-3</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN+47</td>
<td>0.53 (±0.33) X 10^-3</td>
<td>1.2 (±0.2) X 10^-3</td>
<td>0.96 (±0.06) X 10^-3 **</td>
<td>0.17 (±0.07) X 10^-3</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-16</td>
<td>3.0 X 10^-3</td>
<td>7.0 (±2.7) X 10^-3 **</td>
<td>9.4 (±1.6) X 10^-3 **</td>
<td>2.3 (±0.5) X 10^-3 **</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-6</td>
<td>5.5 (±3.1) X 10^-3 *</td>
<td>5.5 (±2.6) X 10^-3 *</td>
<td>8.2 (±0.3) X 10^-3 ***</td>
<td>1.3 (±0.3) X 10^-3 **</td>
</tr>
<tr>
<td>TransIT-LT1</td>
<td>pST0/TLN-7</td>
<td>0.18 (±0.04) X 10^-3</td>
<td>1.3 (±0.3) X 10^-3</td>
<td>0.32 (±0.01) X 10^-3</td>
<td>0.40 (±0.04) X 10^-3</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN+47</td>
<td>0.081 (±0.018) X 10^-3 **</td>
<td>0.40 (±0.40) X 10^-3 *</td>
<td>0.42 (±0.21) X 10^-3</td>
<td>0.23 (±0.12) X 10^-3 *</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-16</td>
<td>1.2 (±0.8) X 10^-3 *</td>
<td>3.6 (±0.8) X 10^-3 **</td>
<td>6.3 (±4.6) X 10^-3</td>
<td>2.4 (±0.2) X 10^-3 ***</td>
</tr>
<tr>
<td></td>
<td>pLHC4/TLN-6</td>
<td>1.8 (±1.1) X 10^-3 *</td>
<td>6.0 (±2.7) X 10^-3 *</td>
<td>7.8 (±7.6) X 10^-3 *</td>
<td>3.2 (±1.0) X 10^-3 **</td>
</tr>
</tbody>
</table>

The luciferase activities shown in Figs. 1 and 2 were divided by the DNA amounts at the same time points shown in Table 1. The values represent the averages (±SD) of three separate experiments, except for one sample (Lipofectamine, pLHC4/TLN-16, 24 hr, n=2). (*P<0.05, **P<0.01, and ***P<0.001 versus pST0/TLN-7).