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日 本 蚕 糸 学 雑 誌

第62巻　第6号（平成5年12月）別刷

Reprinted from

THE JOURNAL OF SERICULTURAL SCIENCE OF JAPAN

Volume 62, Number 6, (December 1993)

日蚕雑 62(6), 523-526 (1993) 短 報
J. Seric. Sci. Jpn.

# Efficient lipofection method for transfection of the silkworm cell line, NISES-BoMo-15AIIc, with the DNA genome of the *Bombyx mori* nuclear polyhedrosis virus

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The silkworm, *Bombyx mori*, cell line (BoMo-15AIIc) (INOUE *et al.*, 1990), susceptible to the *B. mori* nuclear polyhedrosis virus (BmNPV) and to several cytoplasmic polyhedrosis viruses (CPVs), is commonly used for various research purposes such as for the baculovirus-mediated expression of recombinant proteins (KOBAYASHI *et al.*, 1990, 1992), for studies on the interactions between CPVs and BmNPV during their replication *in vitro* and in experiments leading to the development of CPV expression vector. All these studies require the use of highly efficient methods for the transfection of viral genomes into the cells.

Using several mammalian cells, FELGNER *et al.* (1987) demonstrated that the DOTMA {N-[1-(2, 3-dioleyloxy) propyl]-N, N, N-trimethylammonium chloride} mediated transfection (lipofection) method was more efficient for both stable and transient expression of the introduced DNA than either the DEAE-dextran or calcium phosphate-mediated transfection. The increase of the concentrations of DOTMA-containing lipid that improved transfection, however, was toxic and this toxicity varied with the type of cells, the duration of exposure to DOTMA, and the density of cell culture. Therefore, the optimization of the technical conditions of lipofection for each cell line is recommended in order to achieve a high efficacy of transfection. Lipofection method has already been applied successfully for the transfection of *Spodoptera frugiperda* (Sf-9) insect cells with the DNA genome of *Autographa californica* NPV (AcNPV) (PATEL *et al.*, 1992). In order to improve the transfection efficiency and broaden the variety of manipulation techniques for the BoMo-15AIIc cell line, we applied the lipofection method to this cell line.

## Materials and Methods

The DNA genome of the BmNPV P6E isolate (KOBAYASHI *et al.*, 1990) was prepared according to the method described by MAEDA (1989). Transfection of BoMo-15 AIIc cells with BmNPV DNA using the Lipofectin reagent (Gibco BRL) was performed according to PATEL *et al.* (1992) with some modifications.

Lipofectin (8 to 24 $\mu l$) was added to an equal volume of DNA solution containing 1 $\mu g$ of BmNPV DNA and gently mixed. The mixture

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J.K. was an invited scientist at the Institute Armand-Frappier and a recipient of A Long Term Fellowship for Research Abroad from the Science and Technology Agency of Japan. This work was supported in part by NSERC OGP 0003205 grant (Canada).

was incubated for 15 min at room temperature and then added to the wells of a 6 multi-well plate containing $1.5\times10^6$ BoMo-15AIIc cells in 1 m*l* Grace's insect medium (Sigma) lacking FBS (fetal bovine serum) or 1 m*l* MGM-448 medium (MITSUHASHI, 1984) with 10% FBS. The cells were incubated for 4 to 16 hr at 25℃.

Calcium phosphate-mediated transfection was also performed according to MAEDA (1989). 1 $\mu$g of BmNPV DNA was precipitated with calcium phosphate for 30 min at room temperature. The resulting suspension was added to $1.5\times10^6$ BoMo-15AIIc cells in 1 ml MGM-448 medium with 10% FBS. The cells were incubated for 16 hr at 25℃.

After the incubation, the medium containing the lipofection mixture or the calcium phosphate precipitate was removed, and then the cell monolayer was overlaid by 2.5 m*l* of 0.75% SeaPlaque agarose in MGM-448 medium with 10% FBS (INOUE *et al.*, 1990). The plaques were counted from 6 to 10 days after incubation at 25℃.

## Results and Discussion

When the BoMo-15AIIc cells were transfected with the Lipofectin (8 $\mu l$)-BmNPV DNA complex for 16 hr in a serum-free medium according to a lipofection protocol for Sf-9 cells (PATEL *et al.*, 1992), the increase in the number of plaques was about 20-fold compared with the calcium phosphate-mediated transfection commonly used (Table 1). In the presence of growth medium containing serum during the incubation with the Lipofectin-BmNPV DNA complex, the number of plaques was reduced to about 30% of that in the serum-free medium. No significant difference in the transfection efficiency was observed in both serum-free and serum-containing media when the time of incubation was reduced to 4 hr. The increase of the concentration of Lipofectin resulted in the decrease in the number of plaques. In the presence of serum, transfection with both 16 and 24 $\mu l$ of Lipofectin was drastically inhibited and the number of plaques was reduced to less

Table 1. Comparison between lipofections and calcium phosphate-mediated transfection of BmNPV DNA

| Method[1)] | Lipofection reagent ($\mu$l) | Incubation time (hr) | Concentration of serum (%) | Number of plaques | Efficiency of tranfection[2)] |
|---|---|---|---|---|---|
| Lipofection | 8 | 16 | 0 | 428 | 21.4 |
| | 8 | 16 | 10 | 142 | 7.1 |
| | 8 | 4 | 0 | 404 | 20.2 |
| | 8 | 4 | 10 | 145 | 7.3 |
| | 16 | 4 | 0 | 341 | 17.1 |
| | 16 | 4 | 10 | 26 | 1.3 |
| | 24 | 4 | 0 | 332 | 16.6 |
| | 24 | 4 | 10 | 18 | 0.9 |
| Calcium phosphate | — | 16 | 10 | 20 | 1.0 |

1) One $\mu$g BmNPV DNA was used to trasfect monolayer of NISES-BoMo-15AIIc cells as described in Materials and Methods.
2) Ratios calculated relative to the number of plaques formed following calcium phosphate-mediated transfection.

than 10% of those obtained by transfection in serum-free medium.

In this experiment, we demonstrated that a lipofection protocol used for the transfection of Sf-9 cells with AcNPV DNA (PATEL *et al.*, 1992) was also effective in the transfection of BoMo-15AIIc cells with BmNPV DNA and about 20-fold more efficient than the calcium phosphate-mediated transfection method. We also revealed that the same level of efficiency (about $4\times10^2$ plaque forming units per $\mu g$ of DNA) was obtained when the original transfection time of 16 hr was reduced to 4 hr. In mammalian cells, when the duration of the treatment with the lipofectin-DNA complex increased, the transfection efficiency also increased, although prolonged treatment was toxic to the cells (FELGNER *et al.*, 1987). Therefore, the results obtained in this experiment suggest that the optimal duration of the treatment ranged between 4 and 16 hr. We failed to improve the transfection efficiency by increasing the concentration of Lipofectin. This phenomenon may reflect the toxicity of Lipofectin to the BoMo-15AIIc cell line at concentrations higher than 16 $\mu l$. O'REILLY *et al.* (1992) described another lipofection protocol for the transfection of *S. frugiperda* cells with AcNPV DNA using 30 $\mu l$ of Lipofectin for 4 to 5 hours. Therefore, the BoMo-15AIIc cells may be more sensitive to the toxic effects of the Lipofectin reagent than the *S. frugiperda* cells.

Recently, BRUNETTE *et al.* (1992) have reported that lipofection performed in a medium containing serum using the simian kidney cell line, CV-1, was at least as efficient as lipofection performed in serum-free medium, although FELGNER *et al.* (1987) had previously reported that the addition of growth medium containing serum almost completely inhibited transfection in the same cell line. The discrepancy between the results were ascribed to differences in the lipid: DNA ratio, number of cells treated and amount of cationic lipid used. Transfection efficiencies were always higher when the cells were treated with the Lipofectin-DNA complex in a serum-free medium. Interestingly, transfection was more drastically inhibited in a medium containing serum when the cells were treated with higher concentrations of Lipofectin. As suggested in the CV-1 cells, it is likely that the transfection efficiency in the medium containing serum is very sensitive to the amount of cationic lipid used.

The high efficiency of the lipofection method in the transfection of BoMo-15AIIc cells with the BmNPV DNA reported here suggests that this method may be useful for constructing recombinant BmNPVs in the baculovirus expression vector system of *B. mori* and applicable to the transfection of other viral DNAs such as the DNA genome of the granulosis virus, and plasmid DNAs containing foreign genes for transient and/or stable expression.

Until recently, no data of successful transfection of viral dsRNA in cell lines were available. However, RONER *et al.* (1990) succeeded in inducing infectious reovirus in L929 mouse fibroblasts which were lipofected with both reovirus RNA and a rabbit reticulocyte lysate containing reovirus proteins. This achievement, demonstrating that viral double-stranded RNA could be efficiently transfected, led to the application of the lipofection method developed above for the transfection of BoMo-15AIIc cells with the dsRNA genome of BmCPV. This was a necessary step in the development process of CPV expression vector, production of CPV strain reassortants as well as in studies on the host-range specificity of the virus. In preliminary experiments, 10 segments of the BmCPV genome were detected in the extracts of transfected cells by using the lipofection method (data not shown). Based on these results, the

determination of the optimal conditions for the lipofection of BmCPV dsRNA as well as the modification in the protocol to generate infectious reovirus from viral RNA (RONER *et al.*, 1990) for BmCPV is in progress.

**Acknowledgments**

The authors thank Dr. Shigeo Imanishi for providing the MGM-448 medium. The authors thank also Monique Couillard for the technical assistance and Diane Comeau for typing the manuscript.

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