Transfection of rat embryonic cerebral cortical cells with METAFECTENE PRO

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Introduction

In vitro transfection is a useful method to overexpress or knock-down proteins in order to study their role in different cellular mechanisms. Several gene delivery methods are currently used including calcium phosphate, cationic liposome reagent, electroporation, microinjection and viral delivery. Transfection of primary cells, notably of neuronal cells, often results in low transfection efficiency and high cell death. In this study, METAFECTENE PRO, a polycationic liposome reagent, was tested for transfection of rat embryonic cerebral cortical cells with a plasmid expressing the green fluorescent protein (GFP) as a reporter. Optimum lipofection efficiency depends on experimental conditions including cell density, medium components such as serum and antibiotic, quantities of DNA and reagent, time of DNA dilution and complex formation (Dalby *et al.*, 2003). Here, transfection efficiency and cell death were analysed for two different quantities of DNA and three different ratios of DNA:METAFECTENE PRO.

Materials and Methods

Plasmid: Cells were transfected with pCS2-Venus, a plasmid developed by Nagai et al. (2002), which contains a modified GFP, under control of a CMV (CytoMegaloVirus) promoter. Plasmids were extracted and purified using EndoFree plasmid Maxi kit (QIAGEN).

Cell culture: Cerebral cortical cells (400,000; E14.5 Sprague Dawley rats) were plated on poly-D-lysine (0.1 mg/ml) and laminin (16 µg/ml)-coated 25 mm glass coverslips, and incubated overnight in Neurobasal/B27 (Invitrogen) medium containing bovine serum albumin (0.1%), glutamine (2 mM), basic Fibroblast Growth Factor (bFGF, 10 ng/ml), penicillin (50 U/ml), and streptomycin (50 mg/ml). Cells were grown overnight in a humidified 5% CO_2/air incubator at 37ºC.

Transfection: pCS2-Venus plasmid (1 or 3 µg) and METAFECTENE PRO (2 to 24 µl, Biontex Laboratories GmbH, Munich, Germany) reagent were diluted in two different tubes, each containing 200 µl of Neurobasal/glutamine medium. After 20 min of incubation at room temperature, medium containing METAFECTENE PRO was added dropwise to the medium

containing DNA. The mixture was incubated for 30 min at room temperature to allow DNA-lipid complex formation. Meanwhile, cells were washed with Neurobasal/glutamine. Transfection was performed in Neurobasal/glutamine/B27 by adding DNA-lipid complexes dropwise to the cells. After 5 hrs of incubation in a humidified 5% CO_2/air incubator at 37ºC, transfection medium was replaced with plating medium and cells were incubated for 24 hrs. Some coverslips were incubated with 10 µM BromodeoxyUridine (BrdU) for the last 4 hrs of culture.

Cell death evaluation using DAPI staining: 24 hrs after transfection, cells were incubated with DAPI (1 mg/ml) for 10 min before fixation. Only dead cells (that present disrupted cell membrane) incorporated DAPI. Cells were then fixed in ice-cold 4% PFA for 20 min.

Immunocytochemistry: After fixation in 4% PFA for 20 min, cells were labeled either for cleaved caspase-3 (Cell Signaling, 1:400), S-phase marker BrdU (BD Biosciences, 1:100), neural precursor marker nestin (Chemicon, 1:400) or neuronal marker βIII-tubulin (TuJ1, Covance, 1:1000). For BrdU detection, cells were treated prior to immunolabeling with 50 U/ml DNase I for 10 min (Ye *et al.*, 2006). Staining was visualized using Alexa Fluor 594 or 350 conjugated secondary antibodies (Molecular Probes; 1:1000 and 1:400 respectively) and analyzed by fluorescence microscopy with a Zeiss Axiovert 200M inverted microscope and the AxioVision LE Rel. 4.5 software.

Cell counting: Transfected cells and dead cells were counted at 10x and 20x objective respectively on 5 non-overlapping fields on 1 to 3 converslips per group.

Results and Discussion

Cells were grown in conditions promoting proliferation including enriched serum-free medium, bFGF and high cell density. Cells were transfected with pCS2-Venus plasmid at DNA:reagent ratios: 1 µg:2 µl, 1 µg:4 µl, 1 µg:8 µl, 3 µg:6 µl, 3 µg:12 µl, 3 µg:24 µl, in a serum and antibiotic free environment. In these conditions, 24 hrs after transfection, the highest number of cells expressing the GFP reporter protein was obtained with the ratio 3 µg of DNA and 6 µl of METAFECTENE PRO (Fig. 1). Transfection efficiency was about 10% and is similar to the transfection efficiency obtained with Lipofectamine + Plus reagent (Invitrogen) using the same cellular type and culture conditions (Nicot and DiCicco-Bloom, 2001). The cytotoxic effect of METAFECTENE PRO was evaluated with DAPI staining performed prior to fixation and active caspase-3 immunolabeling. The number of dead cells (Fig. 2) and apoptotic (active caspase-3 immunolabeled) cells (Fig. 3) were not significantly different between the different ratios of DNA and METAFECTENE PRO. Some of the transfected cells were stained for BrdU and/or neural precursor nestin or neuronal marker βIII tubulin, indicating that they kept the ability to proliferate or differentiate (Fig. 4).

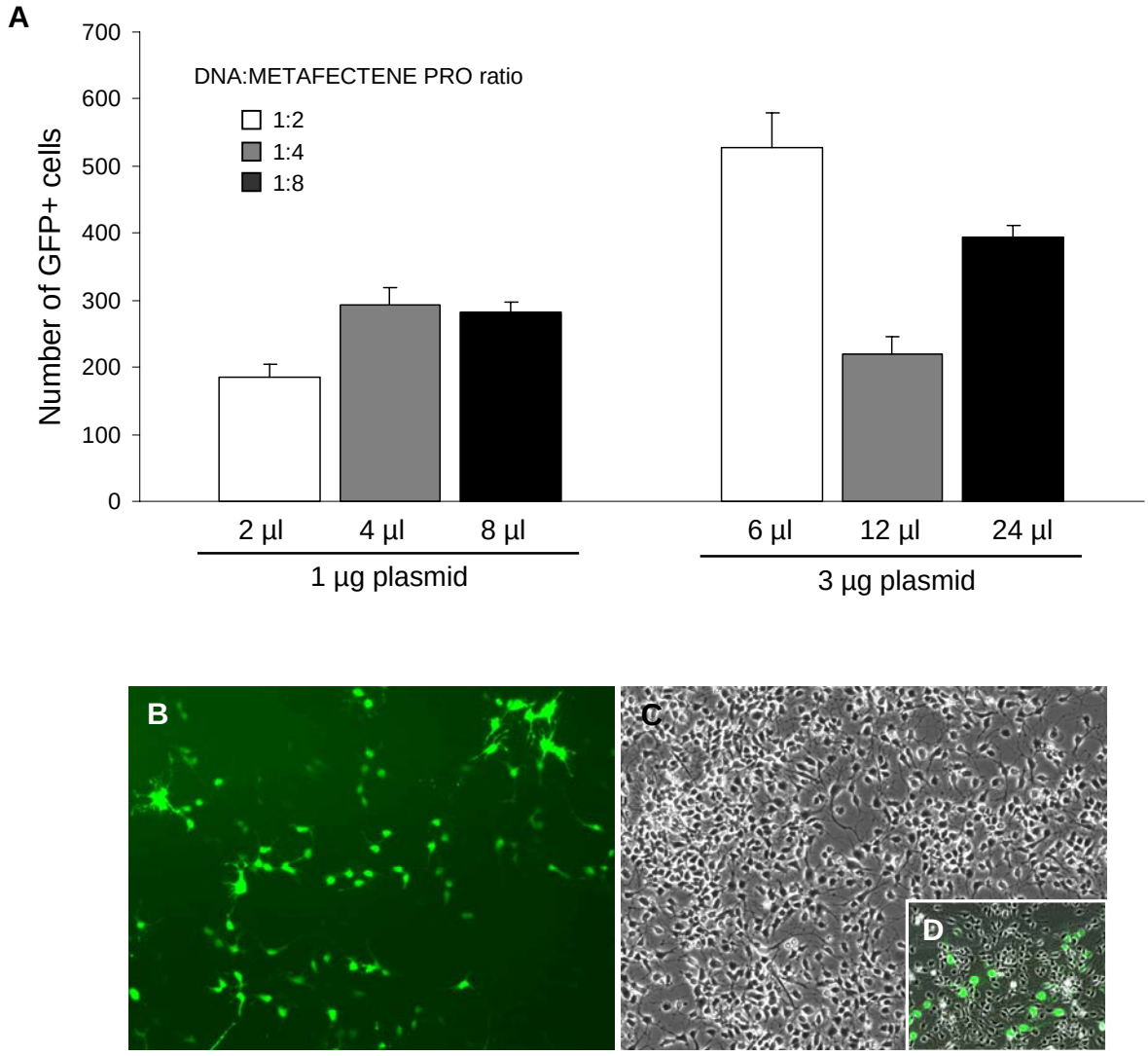

Fig 1: Optimization of DNA:METAFECTENE PRO ratio. Rat embryonic cerebral cortical cells grown overnight were transfected with either 1 or 3 µg of pCS2-Venus plasmid using different volumes of METAFECTENE PRO. A: Twenty four hours after transfection, GFP-expressing cells were counted at 10x on 5 non-overlapping fields. Data represent mean ± SEM obtained from triplicates. B-D: Representative photomicrographs of transfected cells (green fluorescence, B); corresponding cells in phase (C) and merge (D).

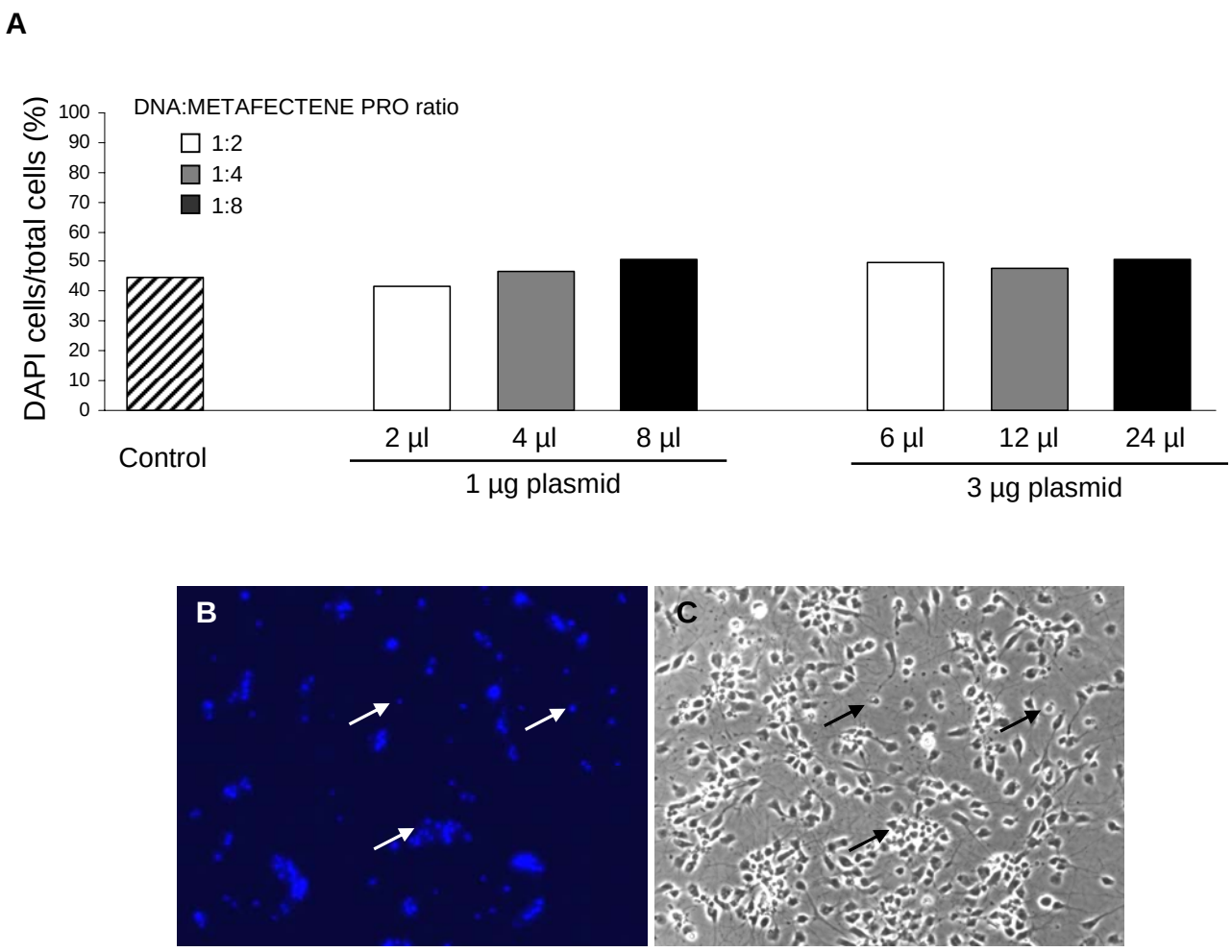

Fig 2: Effect of METAFECTENE PRO-mediated transfection on rat embryonic cerebral cortical cell death. Twenty four hours after transfection, cells were incubated with DAPI prior to fixation. DAPI is only incorporated in dead cells. A: The percentage of DAPI cells (arrows) were counted over the total number of cells. Cells were counted on one coverslip per group. Control corresponds to a coverslip not treated with METAFECTENE PRO. B, C: Representative photomicrographs of DAPI stained cells (blue fluorescence, B) and corresponding cells in phase (C).

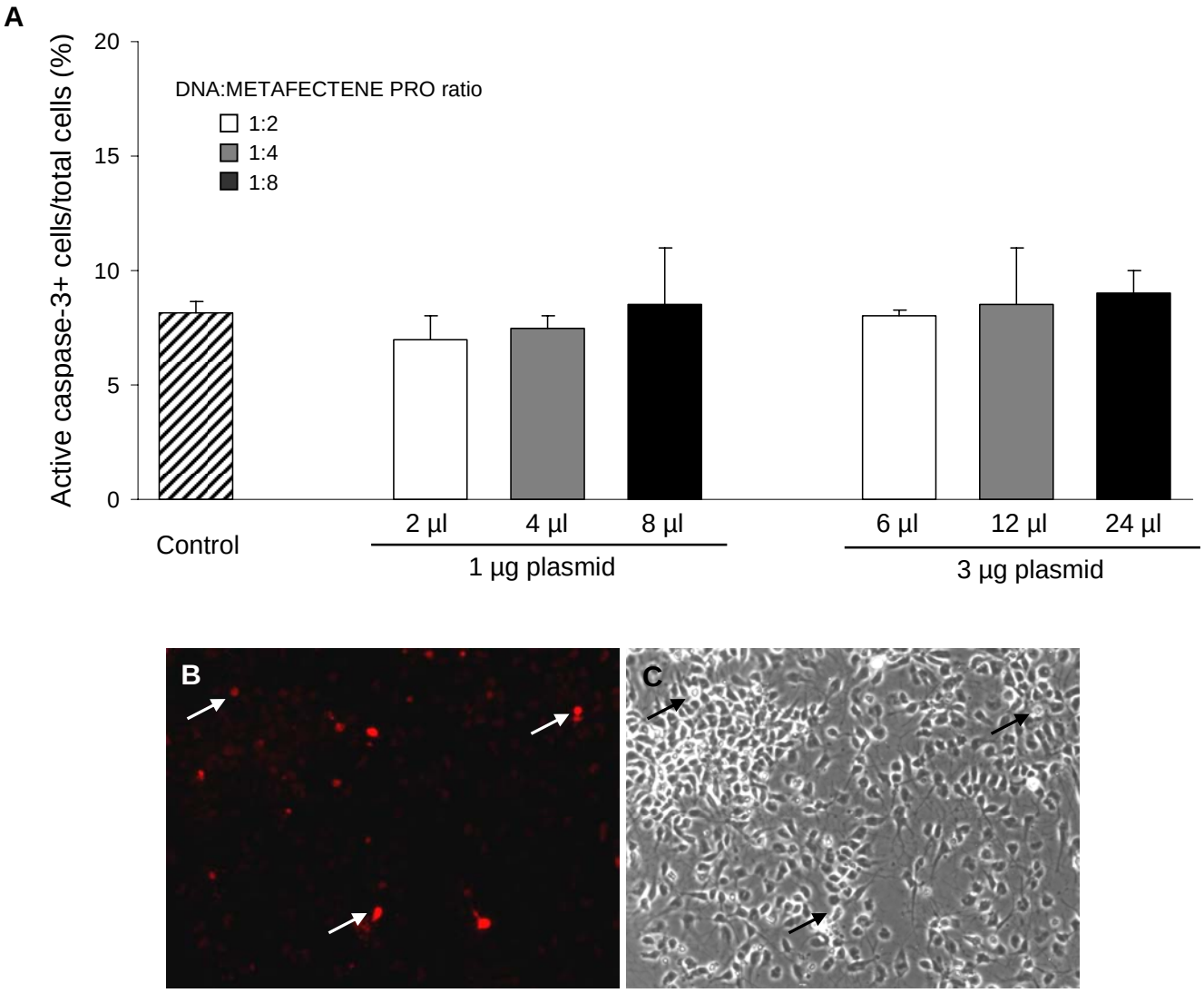

Fig 3: Effect of METAFECTENE PRO-mediated transfection on rat embryonic cerebral cortical apoptosis. Cells were immunolabeled for cleaved caspase-3, 24 hrs after transfection. The percentage of active caspase-3+ cells (arrows) were counted over the total number of cells. Control corresponds to a coverslip not treated with METAFECTENE PRO. Data represent mean ± SEM obtained from duplicates. B, C: Representative photomicrographs of active caspase-3 immunolabeled cells (red fluorescence, B) and corresponding cells in phase (C).

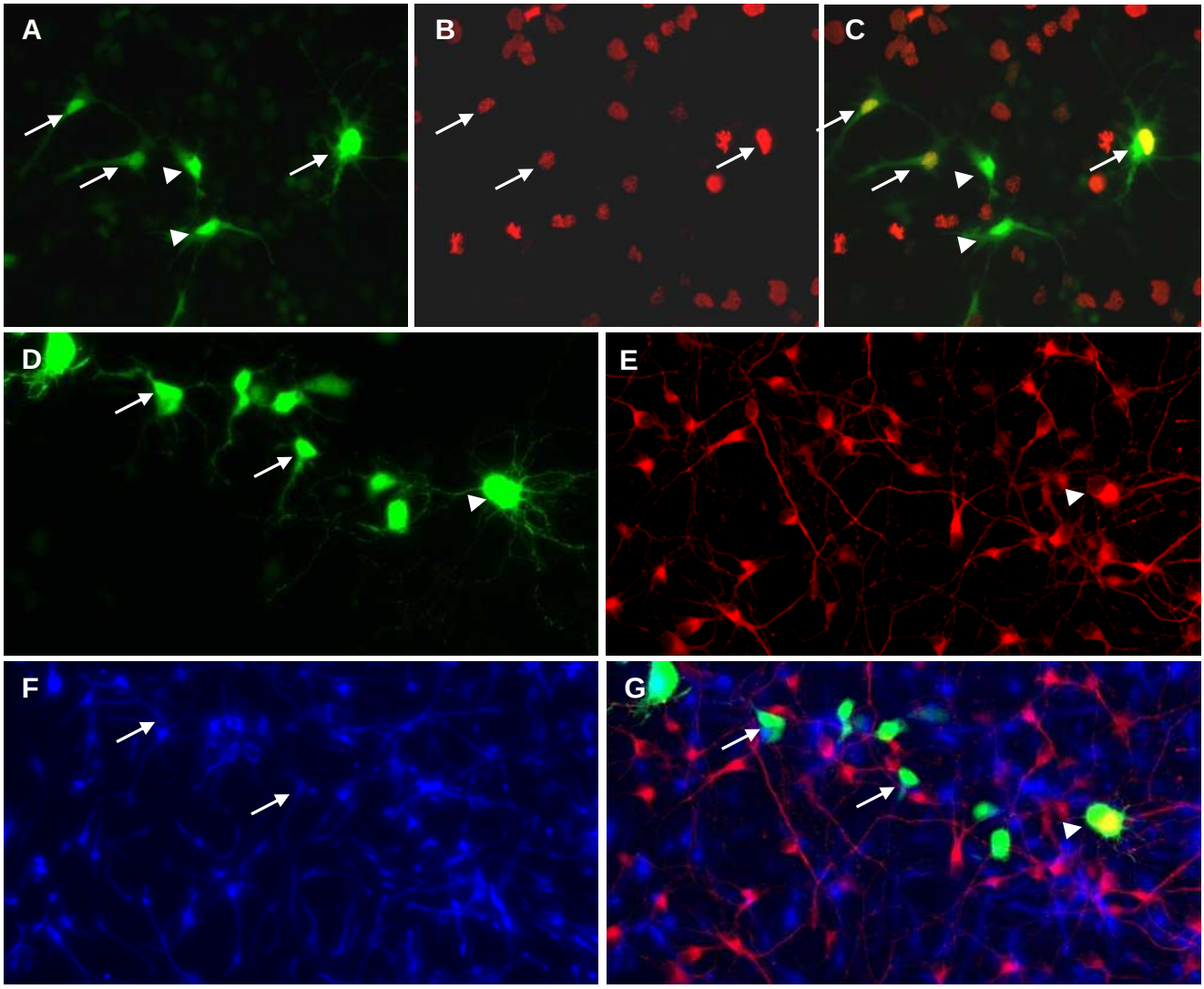

Fig. 4: Characterization of cells transfected with METAFECTENE PRO. A-C: Transfected cells are still able to divide as shown by cells double labeled for GFP (A, green) and BrdU (B, red) (arrows); C: Merge of A and B. Arrowheads point to GFP+/BrdU- cells. D-G: GFP-expressing cells (D, green) express either neuronal marker βIII tubulin (E, red, arrowheads) or neural precursor nestin cells (F, blue, arrows). G: Merge of D, E and F.

Conclusion

In conclusion, METAFECTENE PRO is suitable for transfection of rat embryonic cerebral cortical cells. Since it leads to 10% transfection efficiency in this cell model, it is more appropriate for cell biology studies (morphology analysis, cell death and viability, immunolabeling) than biochemistry analysis. In this study, we tested DNA and reagent concentration variables. Other parameters such as density of cells, components of media, time of culture and complex formation could be improved to enhance the transfection efficiency.

References

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