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An efficient DDAB-mediated transfection of *Drosophila* S2 cells

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ABSTRACT

I have developed an efficient method for transfecting *Drosophila* S2 cells using DDAB, a cationic liposome reagent. The optimized DDAB method resulted in a 10 times or greater increase in transfection efficiency compared with the conventional calcium phosphate method which has been essentially the only way for transfecting S2 cells.

Transfection is an invaluable molecular biological tool to study *in vivo* activities of gene products. A number of different cell lines, mostly of mammalian origin, have been used for this type of study. The Schneider line-2 cell (S2 cell) of *Drosophila melanogaster* was established from primary cultures of 20–24 h embryos more than 20 years ago (1). Depending on the aim of each study, performing transfection experiments using S2 cells can be better than using mammalian cells. The possible advantages are as follows. First, S2 cells provide a null background for studying heterologous gene products [e.g., a mammalian transcription factor Spl (2)]. Second, S2 cells are suitable for the overexpression of heterologous gene products since an average of up to 1000 gene copies are stably inserted in the chromosomal DNA (3). In fact as high as 35 mg of a secreted protein was produced per liter of medium using the inducible metallothionein promoter (4). Third, the functional characterization of putative cell-adhesion molecules has been quite successful (5) since the S2 cells tend to be non-adhesive. Fourth, it is easy to maintain S2 cells since neither trypsin treatment for splitting nor CO_2 supply is required.

The calcium phosphate method has been essentially the only way for transfecting S2 cells (6). However, typically only a few percent of the total cells show expression when transiently transfected (7). To improve the transfection efficiency, I have tried a few chemical agents such as DEAE–dextran, polybrene and dimethyldioctadecylammonium bromide (DDAB) for the transfection of S2 cells. Among them, DDAB (8) was the best and the transfection condition has been optimized by testing the effects of several factors on the transfection efficiency. They are the amount of carrier DNA, the concentration of DDAB, the volumes of DNA, DDAB and M3 media for DNA–DDAB mixture, the incubation period of DNA–DDAB mixture, the media for maintenance and transfection of cells, the cell splitting ratio, the transfection time after splitting, and the cell harvest time.

The optimized transfection conditions are as follows. The S2 cells were maintained in M3 (Shields and Sang M3 insect medium, Sigma) + 10% IMS (insect medium supplement, Sigma) at ~2–4 × 10^6 cells/ml and split 1 to 10 every week. Before preparing cells in a 24-well plate for transfection, DNA–DDAB mixture was prepared. Appropriate amounts (total amount ~100–200 ng) of DNA dissolved in a few microliters of TE were transferred to microfuge tubes. DDAB suspension (250 µg/ml) was mixed with M3 in a 1:2 ratio and 30 µl was transferred to each of the DNA containing microfuge tube and mixed well. While the mixture is sitting in a clean bench, the cell suspension ready for splitting and M3 media were mixed in a 1:4 ratio and 0.5 ml each of the diluted cell suspension was transferred to each well in a 24-well plate. Immediately after plating, the DNA–DDAB mixture was transferred to the well and the cells were incubated for 3 days. Variations in the incubation period of the DNA–DDAB mixture in a range of 10–60 min do not significantly change the transfection efficiency. It usually takes <20 min between preparing the DNA–DDAB mixture and transferring the mixture to the cells when all 24 wells were used for the transfection. Three days after the transfection, the media were removed by aspiration and 20 µl of CLR (cell lysis reagent, Promega) was added to each well. The plate was agitated on a shaker for ~5 min, cell extracts were transferred to microfuge tubes and spun at the maximal speed for 1 min at room temperature, and appropriate amounts of the supernatant were taken for assays. DDAB suspension was prepared as follows. DDAB (Fluka) was suspended in 10 ml of sterile double distilled water (DDW) in a conical tube at 4 mg/ml and sonicated at 15 s intervals until almost fully resuspended with the microprobe of the W-380 sonicator (Ultrasonics Inc.) at the maximal output setting for the microtip. The stock suspension was diluted 10-fold to make 400 µg/ml DDAB and sonicated 10 times at 15s intervals. To make 250 µg/ml DDAB, the 400 µg/ml DDAB was simply diluted with DDW. The remaining stock suspension and appropriately diluted DDAB suspension were stored in 4°C refrigerator and no drastic change in transfection efficiency has been observed for several months. However, it is better to store DNA frozen at high concentration (µg/µl range) and dilute with TE buffer immediately before the transfection.

The transfection efficiencies using the conventional calcium phosphate method and the optimized DDAB method, developed in this study, were compared. A pAc-*lacZ* reporter, which contains a strong distal promoter of the *Drosophila* actin 5C gene (7), was used for transfection. The standardized β-galactosidase activities representing the transfection efficiencies were calculated and the typical results are shown in Figure 1. The DDAB method resulted in 10 times or more efficient transfections than the calcium phosphate method. It was essential to maintain the S2 cells in M3 + 10% IMS medium and transfect the cells in M3 medium for an efficient transfection with DDAB. On the other hand,

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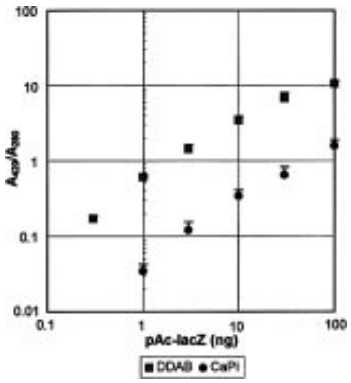

Figure 1. DDAB-mediated transfection of S2 cells is over 10 times more efficient than the conventional calcium phosphate-mediated transfection. DDAB: appropriate amounts (0.3, 1, 3, 10, 30 and 100 ng) of pAc-*lacZ* reporter DNA were mixed with pGeml carrier DNA to make the total amount of DNA for each transfection to 100 ng. Transfection and preparation of cell extracts were performed as described in the text. Two µl of the supernatant was used for the β-galactosidase assay and 5 µl was dissolved in 1 ml DDW and absorbance at 280 nm was measured. The standardized β-galactosidase activities (β-gal activity/protein amount, or A_{420}/A_{280}) are shown. CaPi: appropriate amounts (l, 3, 10, 30 and 100 ng) of pAc-*lacZ* reporter DNA were mixed with pGeml carrier DNA. Total amount of DNA for each transfection was 1000 ng. The M3 + 10% FBS media was used for both maintaining and transfecting cells using 24-well plates. One day after the plating, DNA calcium phosphate co-precipitate was prepared as described previously (7). Two days after the transfection, cell extracts were prepared and assayed as described above.

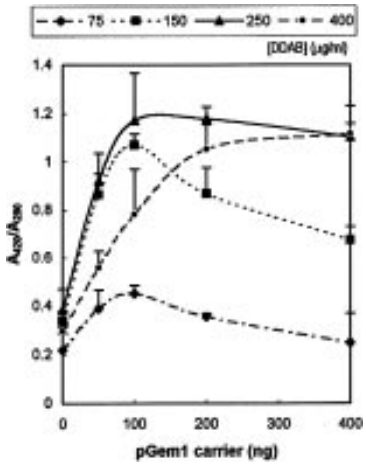

Figure 2. The effects of the amount of total DNA and the concentration of DDAB on the transfection efficiency. Total amount of DNA was varied by adding different amounts (0, 50, 100, 200 and 400 ng) of pGeml carrier DNA to 2 ng of pAc-*lacZ* DNA. Four different concentrations (75, 150, 250 and 400 µg/ml) of DDAB were used.

M3 + 10% fetal bovine serum (FBS) medium was required for both maintaining and transfecting cells when the calcium phosphate transfection method was used. The increased transfection efficiency observed in this study was not simply due to a chance isolation of transfection-competent cells while adapting the cells to the M3 + 10% IMS media because the same cells showed very low transfection efficiency when the calcium phosphate method was used. Nor was it due to DDAB activating endogenous β-galactosidase since no activity was detected when pAc-*lacZ* was omitted.

The effects of the amount of carrier DNA and the concentration of DDAB on the transfection efficiency were studied and the typical results are shown in Figure 2. Various amounts (0–400 ng) of pGeml as a carrier were mixed with non-saturating amount (2 ng) of pAc-*lacZ* reporter DNA for transfection. There are three points. First, as the amount of the carrier DNA increased, the reporter activity also increased as expected, then decreased after a plateau as more carrier DNA was used, which probably reflects that there was a competition between the reporter DNA and the carrier DNA for the DDAB whose amount became limited when too much carrier DNA was used. Second, there is an optimal concentration of DDAB for the maximal transfection efficiency if the amount of the carrier DNA is fixed. This may also be explained similarly, although competition between empty DDAB and DNA-coated DDAB for the limited number of cells may be involved when too much DDAB is used. Third, there is not much variation in the transfection efficiency if the total amount of DNA is between 100 and 400 ng and if the concentration of DDAB is between 150 and 400 µg/ml, suggesting that minor fluctuations in this range are tolerable.

The DDAB method has several advantages over the calcium phosphate method. First, since the transfection efficiency is at least 10 times higher, less DNA, cells, media and cultureware are required. Second, since IMS instead of FBS is required for maintaining S2 cells, it is not only economical but also easier to purify a secreted protein because the concentration of protein in the IMS is low (<l50 µg/ml). Third, unlike other lipids used for transfection, the cost of DDAB is negligible. Fourth, harvesting cells and preparing cell extracts are simplified since neither washing of cells nor sonication is required. The reporter activities in the cell extracts prepared by the classical freeze–thaw method (6) and the current method were comparable. Fifth, the mixing of DNA with DDAB is much simpler and perhaps more reproducible than preparing DNA-calcium phosphate co-precipitate. Sixth, the splitting of cells and the transfection can be performed in the same day and less time-consuming. The transfection method introduced here has been tested only for transient transfection, although it may work well for the stable transfection as well. I have obtained reproducible transfection results using the DDAB method for the last 14 months.

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