Transfection of a lipase-like gene into Drosophila Schneider's 2 (S2) cells with METAFECTENE PRO

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We have research identified a lipase-like gene (LLG) in *Drosophila*. We try to use *Drosophila* S2 cell line to find protein interactions with LLG and the location of LLG in S2 cells.

Materials

METAFECTENE PRO, a liposomal transfection reagent, was obtained from Biontex . The Schneider's *Drosophila* Medium, Fetal Bovine Serum (FBS) and penicillin-streptomycin were obtained from GIBCO. The full-length cDNA of the LLG was cloned into the $CuSO_4$-inducible expression vector pMT/V5 (invitrogen). Different concentration of CuSO4 (sigma) was need to induce protein expression.

Cells

The *Drosophila* Schneider's 2 cell line, derived from *Drosophila* embryos, provides such an expression system and was cultured in Schneider's *Drosophila* Medium (GIBCO) supplemented with 10% FBS (GIBCO), penicillin (GIBCO) (1 U/ml), streptomycin (GIBCO) (1 µg/ml).

Transfection protocol

For transfection, S2 cells were seeded in 2ml Schneider's *Drosophila* Medium at about $2.5x10^6$ viable cells one well of a 6-well plates one day before transfection. The following solution A & B mixtures were prepared in a 12x75-mm sterile tube. Solution A: for each transfection, dilute 2μg of each plasmid DNA into 250μl *Drosophila* serum free medium (SFM) without antibiotics. Solution B: for each transfection, dilute 5μl METAFECTENE PRO into 250μl *Drosophila* SFM without antibiotics. The two solutions were quickly combined by pippetting several times, and incubate for 20 min at room temperature. The cells were washed once with 1 ml of *Drosophila* SFM without antibiotics to completely remove the serum. For each transfection, 0.8ml of *Drosophila* SFM was added without antibiotics to each tube containing the lipid-DNA complexes onto the cells. The cells were incubated for 3~4 hours in a 25℃ incubator. The transfection mixtures were removed and 2ml of *Drosophila* complete medium containing antibiotics were added in S2 cells. The cells were incubated for further 48 hours in a 25℃ incubator. No obvious cell death was observed in the transfection using up to 5μl METAFECTENE PRO.

Western blot

Cell lysate was prepared with RIPA lysis buffer. RIPA lysis buffer: 50mM Tris . Cl (pH 7.4), 1% NP-40, 150mM NaCl and 1 tablet of protease inhibitor cotail (Roche). The S2 cells were centrifuge at 2000rpm for 5 minutes. The supernatant was removed carefully. The S2 cells were washed twice by PBS and centrifuged at 2000rpm for 5 minutes. A mount of 150μl lysis buffer was added to the pellet cells, vortexed gently, and incubated at 4℃ for 30 minutes. The lysed S2 cells were centrifuge at 13000rpm for 25 minutes. The supernatant was transfer to a new tube. A mount of 5μg protein with SDS sample buffer was loaded per well to 10% SDS-PAGE gels. Running for 20 minutes at 50 voltage for the stacking gel and then for 2 hours at 100 voltage when proteins get to separating gel. Proteins in gel were transferred to a nitrocelluse membrane (Amersham) by using 200 mA for 2.5 hours and the membrane was blocked for 1 hour in 5% non-fat milk in TBS-T solution. After blocking, the membrane was washed with TBS-T solution three times every 10 minutes. The first antibody, anti-V5 antibody (1:1000, Invitrogen) was prepared in 1% non-fat dry milk

in TBS-T solution and used to probe the membrane overnight at 4℃. On the next day, the membrane was washed with TBS-T solution three times every 15 minutes. The secondary antibody, goat anti-mouse IgG (1:3000) in 1% non-fat dry milk in TBS-T solution was to probe the membrane for 1 hour at room temperature. Following, the membrane was washed with TBS-T solution three times every 15 minutes, and then incubated with ECL western blotting detection reagents (Amersham). The signal intensity was visualized on the X-ray films.

S2 cell staining

Cover slips containing the cells were placed on ice 5 minutes. The cells were washed on ice with ice-cold PBS three times every 5 minutes. And then the cells were fixed in 4% ice-cold PFA 30 minutes on ice. The cells were washed on ice with ice-cold PBS-T (0.3% triton-X-100) three times every 10 minutes. The cells were stained with anti-V5 antibody (1:500) in 5% bovine serum in PBST at 4℃ for 1 hour. After first antibody staining, the cells were washed with PBS-T three times every 5 minutes. Then the cells were stained with α-mouse cy5 (1:500) at 4℃ for 1 hour. The cells were washed cells with PBS-T three times every 5 minutes.

Results

The S2 cells were transfected with pMT/V5>LLG by METAFECTENE PRO and the expression was induced by adding either 250 or 500μM $CuSO_4$. Western blot analysis indicated a predicted size of LLG protein near 70KD. The expression is $CuS0_4$ dose-dependent (Fig. 1).

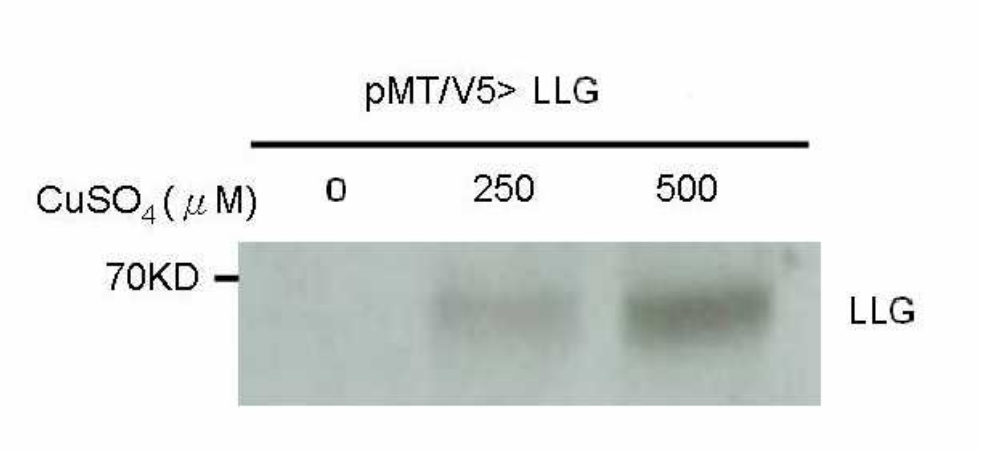

Fig. 1 METAFECTENE PRO mediates the transfection of a LLG into *Drosophila* S2 cells. The molecular weight of LLG is about 70KD. The LLG expression is $CuS0_4$ dose-dependent.

To monitor the transfection efficiency by METAFECTENE PRO in S2 cells, the transfected S2 cells were immune-stained by anti-V5 antibody, visualized Cy5 fluorescence. S2 cell staining reveals the cell transfection does work with METAFECTENE PRO with a transfection rate at about 1/50. The experiment shall be improved by having more repeats. We have tried to use higher concentration of METAFECTENE PRO and found no toxicity to S2 cells.

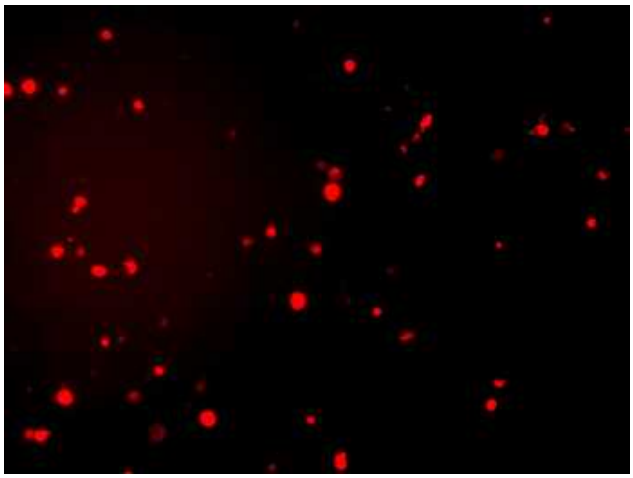

Fig. 2 The cells can be transfected with LGG by METAFECTENE PRO, as evidenced by LGG expression (red).

Conclusion

The protocol for METAFECTENE PRO transfection reagent works in *Drosophila* S2 cells. Moreover, METAFECTENE PRO seems no toxicity to S2 cells under microscope observation.