D-41 Optimization of Condition for Somatic Cell Fusion Between Cabbage Armyworm Fat Body Cell and Primary-Cultured Frog Skin Cell

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Experiments related to fusion between cabbage armyworm, *Mamestra brassicae*, fat body cell line and korean Moodang frog, *Bombina orientalis*, skin cell were performed. Firstly, to select optimal medium and serum concentration for culture of fat body cell line, four different media (TC-100, Grace, TNM-FH, and MM media) and four different concentrations of serum (0,2,5,and 10%) were tested. Of 16 different combinations, 10% FBS and TC-100 was showed the best result. To select optimal medium for cculture of frog skin cells, as the other parental cell, six differents media (MCDB151,F12,MEM, MCDB151:F12<1:1>, MCDB151:MEM<1:1> and F12:MEM<1:1>) were tested. 5% CTS(Charcol Treated Serum) was eually added to each culture medium. Of 6 different media, MCDB151:MEM<1:1> was showed the best result. Using various concentrations of PEG 4000 solution (10,20,30,40, and 50%) and also various DMSO concentrations(0, 5,10,15, and 20%) in 40% PEG 4000 solution, the effect of these solutions on total (homologous and heterologous) atttachment frequency and heterologous attachment frequency which were considered as pre-requisite phenomena of fusion were tested. In case of the total attachment frequency, the result was the best (15%) under 50% PEG solution value, whereas 40% PEG solution gave the highest value (6%) to the heterologous attachment frequency. Survival rates of the 40% and 50% PEG-treated cells were nearly equal as 60%. The effect of DMSO concentrations in 40% PEG solution on fusion was apparent (22%, twice as that of control) for total attachment frequency in case of 10% DMSO but was only slight (5%, 1.2 times as that of control) for heterologous attachment frequency in all the range of DMSO concentrations tested.

D-42 Identification of Alternatively Polyadenylated Form of Fyn

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Fyn is a protein tyrosine kinase originally identified in fibroblasts. Like all known src-family protein tyrosine kinases, fyn has structures essential for membrane association and substrate interaction in the amino-terminal half of the molecule, and the catalytic unit in the carboxyl-terminal tyrosine residue. Until now, two forms of fyn were identified, one is mainly expressed in thymus, Fyn(T), and the other in brain, Fyn(B), which differ in their kinase domains, and are generated by mutually exclusive alternative splicing of a single exon. The fyn(T) plays an important role in T cell signal transduction. Its SH2 domain binds to T cell receptor(TCR) zeta chain and it is also physically associated with CD23. SH3 domain of the fyn(T) mediates binding to phosphatidylinositol 3-kinase in T cells. The fyn mutant experiment implicate fyn as a critical component in TCR signaling in thymocytes and, potentially, in the process that determines T cell repertoire in the adult mouse.

Two mRNAs with different sizes(2.3kb and 3.3kb) for fyn have been noticed by northern hybridization analysis. However, the difference of the two mRNA species has not been resolved yet. We have cloned cDNAs encoding fyn by screening phage expression library using antiserum raised against SH3 domain of PLC-γ. The size of the clone was 3.3kb. DNA sequencing analysis showed that this clone is fyn(T) with 1kb longer untranslated region than the reported usual fyn(T), which suggested that this clone was the one encoding the larger form of fyn mRNA. Northern hybridization analysis using the 3'-end fragment of the clone showed a band of the larger size but not the smaller one. We've found additional polyadenylation signal sequence at the end of this clone. These results suggest that the two mRNA forms are produced by alternative polyadenylation.