2-21. Investigation of post-translational modification of the secreted protein expressed in insect cell lines using baculovirus expression vector system (BEVS)

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In previous experiment, we reported when the heterologous protein is expressed by using baculovirus expression vector system (BEVS), although the amount of intracellular protein is abundant, the amount of extracellular protein is poor. As the link in the chain of the research, we investigated the secretory pathway, important in case of the secretory protein, of the protein expressed in insect cells using BEVS. Firstly, we constructed a green fluorescenct protein (GFP) mutant with a signal sequence of the honeybee mellitin gene in order to visualize the secretion process inside insect cells. The baculovirus vector containing the secretory green fluorescenct protein (sGFP) gene under the polyhedrin promoter was transfected into Sf9 insect cell lines and the recombinant virus (vAc-sGFP) was selected. To investigate the expression pattern of the sGFP and molecular chaperone transcripts, Nortehrn blot anaysis was executed using the total RNA extracted from the cells harvested at multiple days post infection with vAc-sGFP and the cDNA of sGFP and bPDI (*Bombyx mori* protein

disulfide isomerase) as the probe, respectively. The expression of sGFP transcript was shown from 2 days to 5 days post infection (p.i), but bPDI transcript was shown in case of mock-infected and 1 day post infected cell. And we carried out Western blot using the GFP, PDI and calnexin antibody, respectively. .Through the Western blot analysis, we absorved that the GFP protein was expressed from 3 days to 5 days p.i, but PDI and calnexin protein were decreased from 3 days p.i and not detected from 4 days p.i. As the above results, we confirmed that the expression rates of the secteroty protein by BEVS containing the polyhedrin promoter were very poor, because the molecular chaperone was rate-limited expression at the time of the maximum expression of the heterologous protein.