E801 Production of monoclonal antibody against GroES analogous chaperonin of the symbiotic bacteria in *Amoeba proteus*

Jung Eun Lee and Tae In Ahn

Department of Biology education, Seoul National University

GroEx, a heat shock gene analogous to *groE* of *E. coli*, cloned from the symbiotic X-bacteria in *Amoea proteus* contained two open reading frames encoding GroESx and GroELx. When *groEx* was cloned in *E. coli*, the GroESx and GroELx were over 50% of total proteins. Although, unlike those of *E. coli*, GroELx was largely expressed at all temperature by its unique and strong promoters, the level of GroESx was controlled by heat shock consensus promoter and was increased by heat shock. The GroES and GroEL of *E. coli* represent major molecular chaperones that participate in folding and assembly of a variety of proteins. They are also highly expressed in pathogenic bacteria with unknown chaperonin function. Compared with chaperonin function of GroEL (hsp60), that of GroES (hsp10) is less well documented due to the lack of funtional markers. In this study we produced mAbs against the GroESx and used them in identifing the expression of GroESx from X-bacteria in symbiosis. Western blotting analysis of cloned *E. coli* and of *E. coli* itself showed a single protein band of 10kDa, which was increased by heat shock. Immunofluorescence studies on the symbiotic xD strain of *A. proteus* revealed a strong positive signal in synbiotic vacuoles, the nucleus and some weak signal in the cytosol. Besides, a weak signal was also shown in tD Amoeba specially in nucleus and some in the cytosol. Thus, the mAb appeared to be a useful marker to monotor the expression and detect hsp10 anlogues from cloned *E. coli* and other cells.

E802 Heat shock complementation and increase in thermotolerance of *E. coli* by *groEx* cloned from the symbiotic bacteria in *Amoeba proteus*

Ssang Taek Lim and Tae In Ahn

Department of Biology Education, Seoul National University

The *groEx* cloned from endosymbiotic Gram-negative X-bacteria in the xD strain of *Amoeba proteus* has potent intragenic promoters within the coding region of *groESx* in addition to the heat-shock consensus promoters at 5'-extended region of the structural gene. When the gene was subcloned in plasmid vectors, GroESx and GroELx could be highly expressed separately at the temperature above 30°C by these promoters. *groE*$^-$ mutants complemented with the gene formed colony at nonpermissive temperature for the mutant and supported plaque formation for λ phage. Thus, the gene products were found to have chaperonin function in heterologous combination with *groE* gene products of *E. coli*. In growth experiments, *E. coli* transformed with the gene grew to double density of control *E. coli* at 42 and 45°C. Accumulation of both GroESx and GroELx appeared to relieve early growth arrest of *E. coli* at these temperatures and enabled the cells to maintain viability over 50% at 47°C for 8 h. Thus, large accumulation of GroES and GroEL analogues appears to promote the cell growth at 45°C and to confer thermotolerance to *E. coli* at 47°C.