P65

Deletion Mutants of the BiP's ATPase for Regulation Mechanism by a Nucleotide Exchange Factor, BAP

Myoung Joo Lee, Eun Joo Chang and Kyung Tae Chung

Department of Life Science and Biotechnology, Dong-Eui University Busan, Korea

The endoplasmic reticulum (ER) is the site of synthesis, folding, and assembly of secretory pathway proteins, which is controlled in part by the ER Hsp70 family member, BiP. Members of Hsp70 family are highly homologous and regulated by co-chaperones and exchange factors. We identified a mammalian BiP associated protein (BAP) that shared low homology with yeast Sls1p/Sil1, a positive regulator for Kar2p, and cytosolic mammalian HspBP1, a negative regulator for Hsc70. *BAP* encoded an ~54 kD protein with an N-terminal ER targeting sequence, two sites of N-linked glycosylation, and a C-terminal ER retention sequence. It has been studied that BAP binding was affected by the conformation of the BiP's ATPase domain based on in vivo binding studies where BAP bound better to the BiP's ATPase mutants than to wild type BiP, and in vitro, BAP binds better to wild type BiP in the presence of ADP than ATP. Binding of BAP to the ATPase domain of BiP is believed to be important for regulation. However, it has not been elucidated yet which motif of the ATPase domain is associated with BAP for regulation. Therefore, various deletion mutants of the BiP's ATPase were produced in order to investigate binding mechanism. Deletion motives were selected in the ATPase domain by comparing with the ATPase domain of Hsc70, a cytosolic homologue of BiP. Deletion mutant cDNAs were cloned into BamHI과 XhoI sites of a derivative of pSG5 vector. Each deletion mutant were transfected into HEK293 cells and its expression was verified by western blot with rabbit anti-BiP antibody. We are currently investigating which motif of the ATPase is important for binding of BAP.