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BAP, a mammalian BiP associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP

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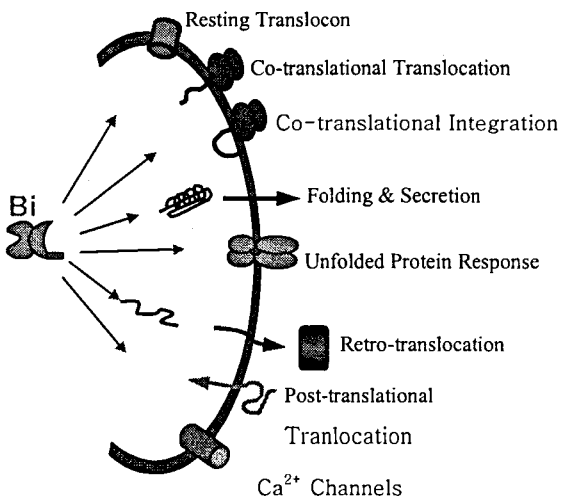
Introduction

The Hsp70 family of molecular chaperones are highly homologous and consist of two distinct domains: a highly conserved N-terminal ATPase domain and a less conserved C- terminal polypeptide binding domain. The chaperone activity of Hsp70 proteins is controlled by the ATPase domain that undergoes a reaction cycle comprised of ATP binding, hydrolysis, and nucleotide exchange. Co-chaperones and cofactors regulate the ATPase domain of Hsp70 proteins. BiP (also known as GRP78) is a mammalian endoplasmic reticulum (ER) homologue of the Hsp70 family. The functions of BiP in the ER are depicted as below;



Recently, a yeast ER protein (Sls1p/Sil1) was isolated from two different genera, which interacts with the ATPase domain of Kar2p, the yeast homologue of BiP. The existence of potential mammalian and invertebrate homologues of Sls1p were reported, but no data are available on their activity.

Purpose :

Identification of potential mammalian regulators of BiPs ATPase activity

Methods :

- Screening Technique: a yeast two-hybrid screen using the ATPase domain of BiP mutant as the bait protein.
- cDNA library: a human liver cDNA library from Clontech.

Results :

- BiP Associated Protein (BAP) was identified.
- BAP was ubiquitously expressed and co-localized with BiP in the ER.
- BAP is a glycoprotein, and its protein level was decreased after tunicamycin treatment.
- The *in vivo* interaction of BAP with BiP is affected by the nucleotide dependent conformational state of BiP.
- BAP stimulates the ATPase activity of BiP.
- BAP promotes nucleotide exchange from BiP.

Conclusion :

BAP represents the first mammalian ER nucleotide exchange factor for BiP

Discussion

All hsp70 family members bind and hydrolyze ATP, and their functions are regulated by the nucleotide bound state. For most hsp70s, co-factors that regulate ATP hydrolysis and nucleotide exchange have been identified. However, until now no regulators of nucleotide exchange for the mammalian ER hsp70 family orthologue had been identified. This study provides the first description of a resident ER protein that serves as a nucleotide exchange factor for BiP.

The amino acid sequence of BAP shows homology with two groups of proteins, Sls1p and HspBP1, that have been implicated in regulating the ATPase cycle of hsp70 proteins. Recombinant BAP protein stimulated the ATPase activity of BiP *in vitro* and caused a further increase in the presence of recombinant ERdj4, suggesting that BAP was more likely to be a functional homologue of Sls1p than of HspBP1. When nucleotide exchange assays were performed under conditions of excess cold ATP, BAP caused the rapid release of labeled ADP from BiP. This characteristic is more similar to results obtained with GrpE, the nucleotide exchange factor present in bacteria and in organelles like chloroplasts and mitochondria, which are thought to be of bacterial origin.

The *in vivo* data for binding of BAP to BiP revealed that more BAP was associated with the ATPase mutants than with wild type BiP, indicating that BAP may prefer the ADP bound form of BiP, which is in keeping with binding data for both Sls1p and Fes1p, a recently identified yeast homologue of HspBP1. In the case of BAP and Sls1p, this should result in the preferential exchange of ADP out of the nucleotide-binding cleft, which explains their positive effects on the ATPase activity of their respective hsp70 proteins. Nucleotide binding experiments demonstrated that BAP does not directly bind either ATP or ADP, so its ability to act as an exchanger for BiP must occur as a result of conformational changes that occur in the ATPase domain of BiP when BAP

binds.

In vivo, it is assumed that ATP must rebind to the nucleotide binding cleft to allow release of bound proteins at the appropriate time so they can fold. This hypothesis is supported by data obtained with BiP ATP binding mutants showing that the mutants prevent the folding of bound substrates but keep them in a soluble form. The *in vitro* addition of ATP to complexes induces the release of BiP. Thus, it is reasonable to assume that the timing or conditions of release might be important and that over-expression of a nucleotide exchange factor could have either positive or negative effects on protein folding. In keeping with the idea of negative effects on protein folding, the over-expression of BAG-1 inhibited Hsp70s ability to refold luciferase and suppressed the positive effect of Hip on Hsp70s chaperone activity. In support of the idea that BAP positively regulates protein folding in the mammalian ER, BAP is expressed highest in tissues like the liver, kidney, and placenta, which produce large amounts of secreted proteins.

In summary, the first mammalian ER nucleotide exchange factor for BiP have been identified, which appears to be a homologue of yeast Sls1p. However, while BAP is highly expressed in secretory tissues, unlike Sls1p, BAP is regulated independent of ER chaperones during ER stress. This suggests that mammalian cells have the ability to inhibit the release of BiP from substrate proteins under conditions that are not conducive to proper folding or assembly.