Examination of calpain and protein kinase/phosphatase systems: Interaction between the post-synthetic protein modifying systems

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The intracellular signaling pathways are regulated by complex networks of enzyme systems. There is a remarkable interplay between the proteolytic and the protein kinase/phosphatase systems. Calpains regulate their target proteins via irreversible limited proteolysis, while protein kinases and phosphatases alter proteins by reversible phosphorylation, turning them into various conformations and functional stages.

In the first part of our work we studied the phosphorylation of calpain B in *Drosophila melanogaster.* We confirmed the presence of the predicted hypothetical phosphorylation sites with *in vitro* experiments. We found that recombinant calpain B can be phosphorylated by PKA as well as by ERK1 and ERK2 kinases. Mass spectrometric analysis revealed that PKA phosphorylates Ser240 and Ser845, in addition, Thr747 was identified as the site of phosphorylation of either ERK1 or ERK2. We employed three independent methods to investigate the effects of phosphorylation on enzyme functions. We demonstrated that phosphorylation increased both of the autoproteolytic activation and the activity of calpain B. Moreover, the kinase treatment enhanced the Ca^{2+} sensitivity of the enzyme. Experiments with phosphorylation mimicking mutants (Thr747Glu and Ser845Glu) supported our previous conclusions. The *in vivo* phosphorylation of Thr747 was detected in EGF stimulated *Drosophila* S2 cells. Our results indicate that ERK signaling pathway activated by extracellular signals results in the phosphorylation and activation of fruit fly calpain B. We also demonstrated the *in vivo* phosphorylation of Ser240 that is presumably not caused by PKA, but by CaMKII. Collectively, our data support the notion that the proteolytic activity of calpain B is regulated by protein phosphorylation/dephosphorylation.

In the second part of our work we investigated the gene of protein phosphatases Z, an important regulatory enzyme in *Candida albicans*. As the first step of the study we proved with Southern blot experiments that, in accordance with data in the literature, *C. albicans* has only one PPZ gene termed *CaPPZ1*. The cloning and sequencing of the *CaPPZ1* of the ATCC 10231 reference strain revealed the polymorphism of the gene and the heterozygosity of the strain. By the analysis of the gene in clinical samples and reference strains we discovered the existence of four distinct *CaPPZ1* alleles. Characteristic nucleotide replacements and insertions/deletions were detected at the coding and noncoding regions. The 3'-noncoding regions of the *CaPPZ1* alleles showed an exceptionally high degree of variability. The sequencing of this area showed that the hypervariable regions from clinical and reference strains fall into one of the previously identified four alleles. The DNA sequencing data were confirmed by RFLP analysis. Altogether we found no more than five *CaPPZ1* allele combinations in different *C. albicans* strains. In addition we detected individual point mutations, which elevated the genetic heterogeneity of this pathogenic yeast. According to our results, the hypervariable region of the *CaPPZ1* gene may be suitable for genotyping *C. albicans* isolated from clinical samples. By comparing the haplotypes of the strains one can reveal the origin of the infection.

Kulcsszavak: *Drosophila melanogaster*, kalcium-függő proteáz, *Candida albicans*, protein foszfatáz. Keywords: *Drosophila melanogaster*, calcium-dependent protease, *Candida albicans*, protein phosphatase