EVIDENCE OF YEAST HSP26 “IN VIVO” PHOSPHORYLATION

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Hsp26 is the best characterized yeast small heat shock protein which possesses co-chaperone activity. Several sHsps have been shown to be phosphorylated *“in vivo”,* like as the sHsps of drosophilas and mammals. Studies of mammalian cells indicate that phosphorylation of sHsps may be an important regulator of their function. However, no phosphorylation of yeast Hsp26 has been shown until this date. Yeast Hsp26 was purified in our laboratory and used to address the question if this sHsp is subject to phosphorylation. We showed that the purified yeast Hsp26 is present as four isoforms of same molecular masses and different isoeletric points and that the preparation is recognized by an anti phosphoserine antibody, implicating in a phosphorylation-mediated isoforms generated from this protein. Although homologous eukaryotic proteins can be isolated in phosphorylated forms and, some of them, dephosphorylated *“in vitro”* by calcineurin, we could not verify any changes in the yeast Hsp26 isoforms, after our assay conditions. In an effort to determine the influence of phosphorylation on yeast Hsp26 chaperone activity and on its oligomerization, it was necessary to design two mutagenic oligonucleotides, with changes on the three phosphorylated residues of the yeast *HSP26* primary sequence. Mutagenesis could be performed using *HSP26* gene, excised from the multicopy pL19, cloned into the pGEM®11Zf(+) vector and the designed mutagenic *“primers”*, using a commercial kit (PROMEGA).

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