EXPRESSION OF A NOVEL STRESS PROTEIN, P66, ASSOCIATED WITH THE PROCESS OF MICROCYST FORMATION INDUCED BY SALT STRESS.

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SUMMARY

Under high salt conditions, haploid myxoamoebae of a true slime mold, Physarum polycephalum retracted their pseudopodia and changed their shape into disk-shaped form, then they constructed the cell wall to form microcysts. These morphological changes of haploid myxoamoebae were associated with changes of the distribution of actin filaments in the cells. Staining with phalloidin showed that actin filaments were uniformly distributed throughout the cytoplasm of the myxoamoebae. The actin structures changed into short rods under the stress conditions associated with the appearance of disk-shaped cells.

Several proteins were induced under the stress conditions, among which a 66k-protein, p66, was most prominently induced. However, p66 was not induced, when diploid plasmodia of the same species were exposed to stress conditions.

This stress protein was co-precipitated with polymerized actin and bound to ATP. A double staining of the disk-shaped cells with phalloidin and anti-p66 antibody revealed superimposable localization of the p66 and actin filaments in the short rods. p66 was immunologically unrelated to the common stress proteins, HSP70 and HSP90, those are highly conserved during evolution.

In order to know the structure and the regulation of its expression, cDNA for p66 was cloned and its nucleotide sequence was determined. From its deduced amino acid sequence, p66 is considered to be a novel stress protein.

A 42k-protein, p42, was found to form a complex in vivo with actin and p66, and it is considered to act as a regulatory factor in the binding of p66 and actin.

To get further information, full length of p66-cDNA and p42-cDNA have been prepared and their nucleotide sequencing are now under way.