

Signaling pathways for encystment and protein phosphorylation in *Colpoda cucullus*

Yoichiro SOGAME¹, Eiji KINOSHITA², Katsuhiko KOJIMA³, Toshikazu TAKESHITA³ and Tatsuomi MATSUOKA¹

(¹Inst. Biol. Sci., Kochi Univ., ²Dept. Funct. Mol. Sci., Grad. Sch. Biomed. Sci., Hiroshima Univ.,

³Dept. Microbiol. Immunol., Sch. Med. Shinshu Univ.)

SUMMARY

Encystment of *Colpoda cucullus* was induced, and the morphogenetic transformation was preceded by an enhancement of the *in vivo* phosphorylation level of several proteins. Immunofluorescence microscopy using anti-phosphoserine antibody showed that these phosphorylated proteins were mainly localized in the macronucleus and the cell cortical region. SDS-PAGE and biotinylated Phos-tag/ECL of isolated macronuclei showed that a 33 kDa protein (p33) was localized within the macronuclei. LC-MS/MS analysis showed that the p33 may have been ribosomal P0 protein, and its function is presumably to assist in various macronuclear events during encystment, such as chromatin reorganization. In the early stage of encystment, a 50 kDa protein (p50) disappeared, while the expression of a 49 kDa protein (p49) was enhanced. In the presence of puromycin or actinomycin D, the modification in the expression of these proteins was cancelled, suggesting that the encystment-dependent alteration of these proteins is involved in a transcriptional regulation. The LC-MS/MS analysis of the p50 and p49 isolated by 2D-PAGE or 1D-SDS-PAGE revealed that p50 and p49 are mitochondrial ATP synthase b chains and elongation factor 1a (EF-1a), respectively. The EF-1a may be involved in cytoskeletal reorganization during encystment.