Analysis of two types of IP39 proteins from *Euglena gracilis* using specific peptide antibodies

Kyohei TAKEUCHI[1], Yasutaka SUETOMO[2] and Toshinobu SUZAKI[1]
([1]Dept. Biol., Grad. Sch. Sci., Kobe Univ., [2]Iwakuni City Microlife Museum)

SUMMARY

The plasma membrane of *Euglena gracilis* possesses a densely-arranged array of intra-membrane proteins, IP39, which are implicated in the mechanism of rounding-up of the cell (euglenoid movement). Molecular cloning of cDNA encoding IP39 has been carried out and two types of cDNA were identified. In this study, anti-peptide antibodies were generated against deduced amino acid sequences of the two types of IP39 proteins, α- and β-IP39, to examine molecular arrangement and cellular localization of these protein isoforms. Immunofluorescence microscopy showed that both α- and β-IP39 are uniformly localized around the cell cortex. Immunoblot analysis showed that both types of IP39 exist in the membrane fraction in either monomeric or dimeric form. SDS-PAGE of IP39 in the presence of various concentrations of β-mercaptoethanol suggested that disulphide bonding is not involved in the dimerization of IP39. Analysis of phosphorylation levels of IP39 showed that IP39 is constitutionally phosphorylated, regardless of whether IP39 proteins were prepared from elongated or rounded cells, indicating that protein phosphorylation is not involved in euglenoid cell shape change.