CHARACTERISTIC VARIATION OF CLONED ENDOSYMBIOTIC ALGAE IN PARAMECIUM BURSARIA.

N.Nishihara[1], M.Murata-Hori[1], T.Yamada[2], T.Kosaka[1] and H.Hosoya[1].

[1]Department of Biological Science, Faculty of Science, [2]Faculty of Engineering, Hiroshima University, Higashi-Hiroshima.

Paramecium bursaria (green paramecium) has many endosymbiotic algae in its cytoplasm. Symbiotic algae are very similar to the genus Chlorella in morphology. The two partners in the symbiosis can be separated, extracellularly cultured and re-established the symbiotic relationship. We have reported that algae-free parameciawere obtained by the exposure to the herbicide paraquat (Zool. Sci., 12: 807-810, 1995), and several algal cloneswith high infectivity to algae-free paramecia were established and cultured in CA medium (Protoplasma, 203:in press, 1998). Here, we compared the morphological characteristics and re-infectivities of several cloned symbiotic algae from several strains of P.bursaria. Further, partial sequences of the small subunit rRNA encoding gene (SSUrDNA) of cloned algae were amplified using polymerase chain reaction (PCR). When the amplified PCR fragments were compared with each other, several differences in DNA sequences were observed between infective and noninfective cloned algae. This result suggests that species of symbiotic algae in P.bursaria was not single.

Isolation of myosin light chain kinase(s) genes from HeLa cells

T. Iwasaki, K. Ohno, H. Oohara, M. Murata-Hori and H. Hosoya. Dep. of Biol. Sci., Fuc. of Sci., Univ. of Hiroshima, Hiroshima.

We are trying to identify the kinases which phosphorylate the myosin II regulatory light chain in HeLa cells. For this purpose, we screened of a HeLa cDNA library under the low stringency with a horse radish peroxidase-labelled *SalI-HindIII* cDNA fragment of the bovine stomach myosin light chain kinase (MLCK) gene. This fragment encodes the kinase and the calmodulin-binding domains. As the result of this, we could obtain some novel protein kinases.

EFFECT OF WORTMANNIN, A MYOSIN LIGHT CHAIN KINASE INHIBITOR, ON FIRST CELL DIVISION OF SEA URCHIN EGGS

Shuichi Masuda and Hiroshi Hosoya. Department of Biological Science, Faculty of Science, Hiroshima University, Higashi-Hiroshima, 739-8526

We investigated effects of wortmannin, a specific inhibitor of myosin light chain kinase (MLCK), on first cell division of sea urchin eggs to analyze roles of MLCK on cell division. Wortmannin, found as an antifungal antibiotic, inhibits phosphatidilinositol 3-kinase activity at the concentration of 10^{-9} M and MLCK activity at the concentration of 10^{-6} M. Here, we showed that wortmannin inhibited cell division at the concentrations of 2.3 mM but not 0.23 mM. When wortmannin was added to the sea urchin embryos during interphase, mitotic apparatus was not formed. Addition of wortmannin at anaphase inhibited cytokinesis but not formation of cleavage furrow. These data strongly suggest that MLCK activity has an important role in the cell division through the both formation of mitotic apparatus and contraction of cleavadge furrow.

PURIFICATION AND CHARACTERIZATION OF CASEIN KINASE II IDENTIFIED AS MRLC KINASE FROM SEA URCHIN EGGS

S.Komaba, H.Hajime and H.Hosoya. Department of Biological Science, Faculty of Science, University of Hiroshima, Hiroshima.

Ca^{2+}-independent myosin regulatory light chain (MRLC) kinase was purified from eggs of sea urchin (*Hemicentrotus pulcherrimus*). The kinase was identified as casein kinase II (CK-II) composed of α and β subunits by analysis of partial amino acid sequenses. Purified CK-II phosphorylates threonine residue of MRLC. 2-dimentional phosphopeptide mapping showed that the phosphorylation site of MRLC was different from both MLCK site and PKC site. These results suggest that CK-II may play an important role in the regulation of myosin II activity during the development of sea urchin.

HEAT SHOCK PROTEIN HSP27 LOCALIZED SPINDLE FIBER AND INHIBITED POLYMELIZATION OF TUBULIN

K. Kurogi[1], M. Hino[1], M. Murata-Hori[1], M, Okubo[1], T. Nishikata[2], and H. Hosoya[1]

[1]Dept. of Biol. Sci., Fac. of Sci., Hiroshima Univ., Higashi-Hirosima, 739, japan. [2]Dept. of Biol. Sci., Fac. of Sci., Konan Univ., Kobe, 658, japan.

To elucidate the mechanism of cell division, we are investigating proteins specific to mitotic or interphasic cells using monoclonal antibodies. Here we raised several monoclonal antibodies against mitotic HeLa cells. One of them, designated as mH3 (mitotic HeLa 3), recognized a 27 kDa component in mitotic and interphase HeLa cell extract. By using mH3, a cDNA encoding the gene for mH3 antigen was obtained. The nucleotide sequence of the cDNA has been identified as taht of human heat shock protein HSP27. We further showed that (1)indirect immunofluorescence observations showed that HSP27 localized filamentous network in interphase cells. In mitotic cells, the network disappeared and HSP27 localized spindle fiber, and (2)HSP27 inhibited polymerization of tubulin in vitro.

Analysis of CaM kinase I homolog from HeLa cells

H.Oohara, M.Murata-Hori and H.Hosoya Dept. of Biol. Sci., Fuc. of Sci., Hiroshima Univ., Higashi-Hiroshima, 739-8526,Japan

The phosphorylated myosin II regulatory light chain (MRLC) is thought to play a critical role for cytokinesis. However, little is known about the protein kinases which phosphorylate MRLC during cell division. It is important to identify kinases which control the phosphorylation of MRLC during cytokinesis. It is known that CaM kinase I phosphorylates MRLC *in vitro*. Here, we isolated CaM kinase I homolog gene from HeLa cDNA library. We are investigating whether this kinase has an important role for the phosphorylation of MRLC during cell division.