［課程－２］

審査の結果の要旨

氏名 ラトナ ワユニ

EhRab7D, a small GTPase previously identified from isolated phagosomes, was hypothesized to have function in trogocytosis (internalization of the prey by nibbling) and phagocytosis, and thus in pathogenic mechanisms. To characterize the biological role of EhRab7D, overexpression and gene silencing *E. histolytica* strains were established, and assays for trogocytosis, phagocytosis, pinocytosis, motility, cellular adhesion, as well as RNA seq analysis, were performed using these strains. EhRab7D interacting proteins were identified using GST-EhRab7D in affinity purification then *in silico* analysis of EhRab7D protein and prediction of its secondary structure were also performed to discover its unique features among EhRab7 isotypes. The main results of the study as follows:

1. EhRab7D was found to localize to small vesicles in the cytosol, but not trogo- and phagosomes.
2. Repression of *EhRab7D* gene expression by gene silencing caused enhancement of trogocytosis and phagocytosis of CHO cells, while overexpression of EhRab7D caused reduction of the ingestion.
3. Repression of *EhRab7D* gene expression by gene silencing caused reduction of motility and cellular adhesion.
4. Repression of *EhRab7D* gene expression by gene silencing caused enhancement of pinocytosis.
5. Twenty-seven proteins were identified as effector candidates of EhRab7D through affinity purification. Among 27 candidates, 11 of them were related to actin including Arp2/3 protein. These effector candidates interact with EhRab7D in GDP-dependent manner.
6. Affinity purification of GST-EhRab7D did not only show proteins related to vesicular traffic and cytoskeletal rearrangement, many metabolism-related proteins were identified to interact with EhRab7D-GDP. These proteins include phosphoglycerate, acetyl-CoA synthetase, cysteine synthase, glyceraldehyde-3-phosphate dehydrogenase, malic enzyme, and oxidative stress related protein (thioredoxin reductase). Some metabolic proteins were also found to bind to

EhRab7D-GTP including alcohol dehydrogenase 3, elongation factor 1-alpha, and oxidative stress related proteins (peroxiredoxin).

7. *In silico* analysis of EhRab7D identified a unique acidic amino acid stretch composing the natural disordered region in the carboxyl terminus. In addition, amino acid substitutions within the previously identified effector binding surface of mammalian Rab7 were identified.

In this study, role of EhRab7D in actin-related processes (trogocytosis, phagocytosis, pinocytosis, motility and cellular adhesion) has been investigated in *E. histolytica*. Rab7 small GTPases are involved in endosome/phagosome maturation and widely conserved among eukaryotes and known to regulate membrane traffic for lysosome biogenesis. Thus, my findings add novel insights into the role of Rab7 as a regulator of actin cytoskeleton.

よって本論文は博士（保健学）の学位請求論文として合格と認められる。