J. Chosun Natural Sci.
Vol.16, No.2 (2023) pp.69-74
https://doi.org/10.13160/ricns.2023.16.2.69

Roles of RasW in Cell Morphology, Migration, and Development in Dictyostelium

Nara Han and Taeck Joong Jeon†

Department of Integrative Biological Science & BK21 FOUR Educational Research Group for Age-associated Disorder Control Technology, Chosun University

Abstract

In *Dictyostelium*, there are 15 Ras subfamilies, including 11 Ras, 3 Rap, 1 Rheb. The Ras proteins are involved in regulating various cell processes as switch proteins. The functions of many Ras proteins have been identified, but some of Ras proteins have not yet been identified. Here, we focused on identifying the roles of RasW among them. To investigate the functions of RasW in cell morphology, cell migration, and development in *Dictyostelium*, we compared the phenotypes of wild-type cells and *rasW* null cells. *rasW* null cells showed a larger, more spread-out morphology and reduced cell motility compared to wild-type cells. There was no significant difference between wild-type cells and *rasW* null cells during multicellular developmental process. These results suggest that RasW is involved in regulating cell morphology and cell migration in *Dictyostelium*.

Keywords: Ras proteins, RasW, Cell migration, Development, *Dictyostelium*

(**Recceived** May 26, 2023; **Revised** June 12, 2023; **Accepted** June 20, 2023)

1. Introduction

Dictyostelium discoideum is the cellular slime mold that known as free-living social amoeba. All of *Dictyostelium* have a completely sequenced haploid genome[1]. *Dictyostelium* has the same cellular structure and intracellular signaling as mammalian cells. It is also simple to genetically manipulate, has a short life cycle, and has many phenotypes that encode many human orthologues. Thus, it has been used as a model organism for study of eukaryotic cell biology, including cell migration, cytokinesis, and the Ras signaling pathway[2].

Dictyostelium has a unique life cycle. In nutrient-rich conditions, *Dictyostelium* cells live as single cells, but upon starvation, the cells release the chemoattractant cAMP. Through migration toward to chemotactic orientation, about 100,000 cells aggregate to form a multicellular mound[3,4]. Then the multicellular mounds differentiate into elongated migrating slugs. Finally, slug cells divide into two cell populations, prestalk and prespore, to form fruiting bodies. The prestalk cells

† Corresponding author: tjeon@chosun.ac.kr

differentiate into stalks that support the spores, and the prespore cells differentiate into spores, which return to unicellular life when the nutritional environment improves[5,6].

In *Dictyostelium*, there are 11 Ras, 3 Rap, and 1 Rheb[7]. Ras proteins are small GTPases that become activated when GTP is bound by GEFs (Guanine nucleotide exchanging factors) and become inactivated when GDP is bound by GAPs (GTPase-activating proteins)[8]. These Ras proteins act as switches and affect the regulation of various cellular processes including cell motility, cytokinesis, and multicellular development in *Dictyostelium*[7,9]. Many Ras proteins have been characterized their functions in cell migration and development, but some of Ras proteins including RasW have not studied yet. Therefore, we investigated the functions of RasW in diverse cellular processes by examine phenotypes of *rasW* null cells.

2. Materials and methods

2.1. Strains and cell culture

Dictyostelium cells such as wild-type KAx-3 cells (DBS0236487) were obtained from the *Dictyostelium* Stock Center (DictyBase) and knock-out strain was obtained from National Bio Resource Project Cellular slime molds (NBRP Nenkin). All the cells were grown in HL5 medium at 22℃. The knock-out strain was cultured in presence of 10 μg/mL of blasticidin or G418.

2.2. Development

Development was performed as described previously[10]. Exponentially growing cells were harvested and washed twice with 12 mM Na/K phosphate buffer (pH 6.1) and resuspended at a density of 3.5 x 10^7 cells/mL. 50 μL of the cells were placed in non-nutrient Na/K phosphate agar plates and developed. The multicellular organisms were examined and captured with a phase-contrast microscope.

2.3. Random motility

Random motility was performed as described previously[11]. Fully grown cells in a 100 mm plate were washed with HL5 medium and resuspended with 10 mL of HL5 medium. Then, 3 mL of HL5 and 50 μl of resuspended cells were added to a 3 mL plate and adhered for 30 minutes. Adherent cells were washed twice with Na/K phosphate buffer and photographed after incubation for 30 minutes. The images of migrating cells were taken at time-lapse intervals of 1 minutes for 30 minutes using an inverted microscope (IX71; Olympus) with a camera (DS-Fil; Nikon).

2.4. Analysis of random motility

The data of cell migration in random motility were analyzed using NIS-Elements software (Nikon) and ImageJ software (National Institutes of Health). The trajectory speed was calculated by dividing the total distance that traveled of a cell with time. The data were collected from three independent experiments and expressed as the means ±standard deviation (SD).

3. Results

To investigate the function of RasW in *Dictyostelium*, we first performed the computer anal-ysis of RasW. *Dictyostelium*

RasW (DDB_G0270122) is composed of 216 amino acids and contains a Ras domain at the N-terminal region. The expected molecular mass of RasW is approximately 24.8 kDa. Phylogenetic analysis of Ras proteins showed that RasW is closet to RasV.

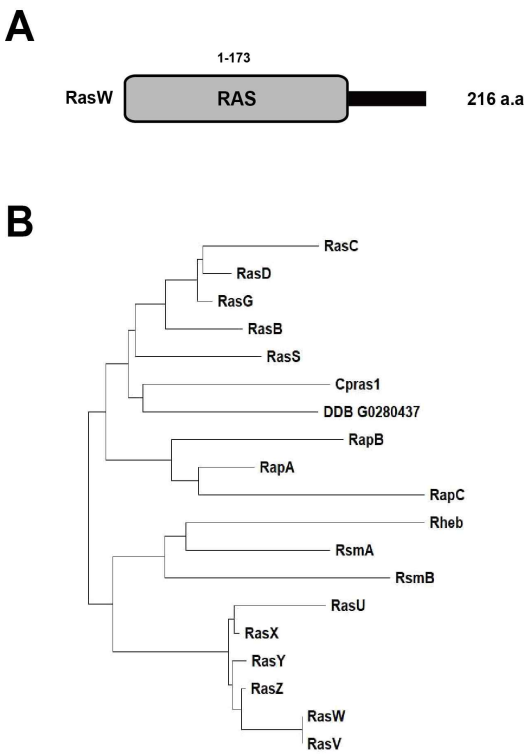

Fig. 1. RasW domain structure and phylogenetic analysis. (A) Domain structure of *Dictyostelium* RasW protein. (B) Phylogenetic tree of Ras proteins. The amino acids of Ras domains were aligned by ClustalW and a phylogenetic tree was drawn to see the homology among RAS domains.

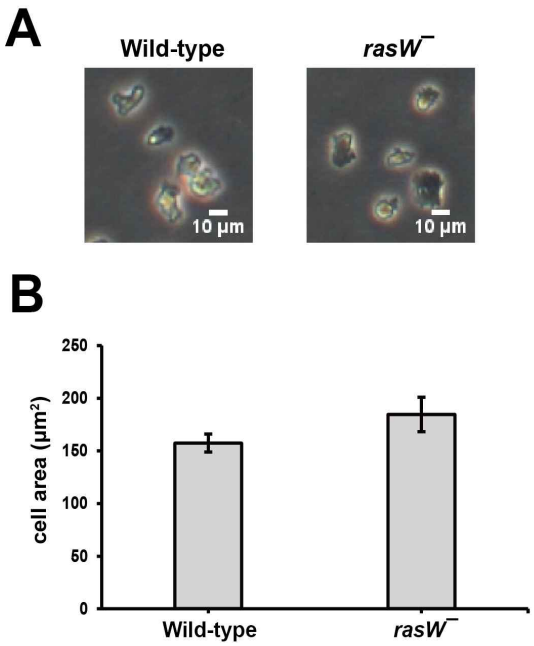

Fig. 2. Cell spreading of *rasW* null cells. (A) Morphology of the cells. The morphologies of the cells were captured and compared to those of wild-types cells. (B) Quantification of the cell area. The area of cells was measured using imageJ software and graphed. The values are the means ±SD of at least three independent experiments.

Next, we compared the morphological characteristics of wild-type cells and *rasW* null cells to determine the role of RasW in regulating cell morphology. *rasW* null cells had larger and spread morphology compared to wild-type cells. Quantification of the cell area showed that the mean size of *rasW* null cells was approximately 2-fold larger than wild-type cells(Fig.2). These suggest that RasW plays a role in maintaining cellular morphology.

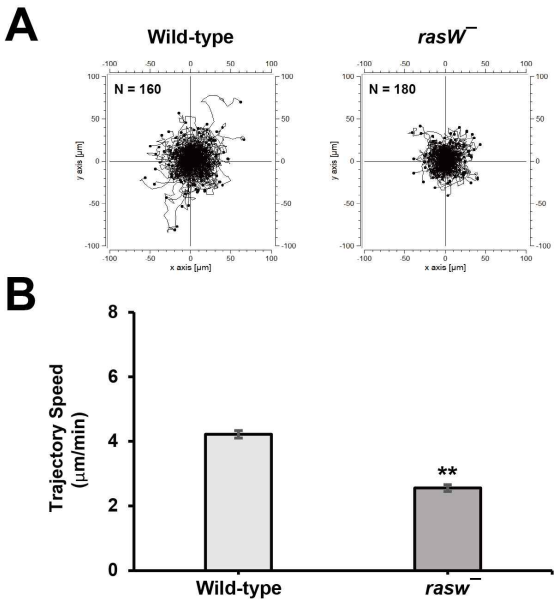

Fig. 3. Random motility. (A) Trajectory of cells. (B) Quantification of cell motility. Trajectory speed indicates the speed of the cell movement. Error bars represent ±SEM of three independent experiments (**p<0.01 compared to the control by the student's t-test).

On the other hand, to determine the roles of RasW in cell migration, we examined the motility of the wild-type cells and *rasW* null cells in the absence of chemoattractant. Wild-type cells showed a speed about 4 μm/min, and *rasW* null cells showed a reduced migration speed of 2~3 μm/min compared to wild-type (Fig.3). This suggests that deletion of RasW negatively affects cell migration.

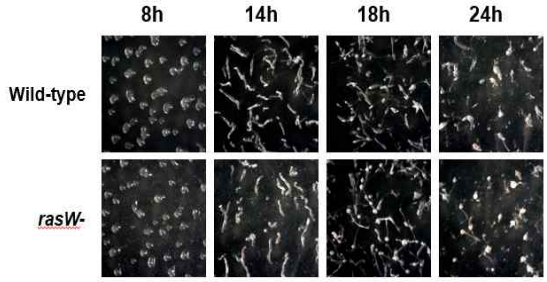

Fig. 4. Developmental phenotypes of *rasW* null cells. Exponentially growing cells were development on non-nutrient agar plates. Representative developmental images at the developmental stages were presented.

Additionally, we compared the developmental phenotypes of wild-type cells and *rasW* null cells to investigate the effects of RasW in developmental processes. As a result, upon starvation, both wild-type cells and *rasW* null cells aggregated to form multicellular mounds, slugs, and fruiting bodies. In these processes, both wild-type cells and *rasW* null cells formed a single tip from a mound. There was no temporal or morphological difference between them. This suggests that RasW does not affect to developmental processes.

4. Discussion

This study provides evidence for the functional role of RasW in cell morphology, cell migration, and multicellular development in *Dictyostelium*. *rasW* null cells showed larger, more spreading morphology and reduced migration speed compared to wild-type cells. However, no significant temporal and morphological differences were observed between wild-type cells and *rasW* null cells during multicellular development. These results suggest that RasW functions to suppress the

spread of cell morphology and activate cell migration particularly in the absence of chemoattractant.

The RasW knockout cells used in our study were produced using the restrictions enzyme- mediated integration (REMI) technique. Therefore, we designed additional experiments. By overexpressing RasW into *rasW* null cells, we will confirm to determine whether the observed phenotypic differences are directly affected by the absence of RasW function. This experiment will provide further insight into the functional role of RasW in these cellular processes.

Many Ras proteins known to suppress the spreading of cell morphology are almost associated with weak adhesion[12]. Interestingly, although not shown in this study, we observed that *rasW* null cells had very weak adhesion. Furthermore, when culturing *rasW* null cells on 100 mm plates, we observed a phenotype that some cells failed to attach and floated when the cell density exceeded a certain threshold. These suggest that RasW may be involved in the quorum sensing.

References

[1] L. Eichinger, et al., A. Kuspa, and P. H. Dear, "The genome of the social amoeba *Dictyostelium discoideum*", Nature., Vol. 435, pp. 43-57, 2005.

[2] J. Martín-González, J. F. Montero-Bullón, and J. Lacal, "*Dictyostelium discoideum* as a non-mammalian biomedical model", Microb. Biotechnol., Vol. 14, pp. 111-125, 2021.

[3] S. S. Willard and P. N. Devreotes, "Signaling pathways mediating chemotaxis in the social amoeba, *Dictyostelium discoideum*", Eur. J. Cell Biol., Vol. 85, pp. 897- 904, 2006.

[4] H. C. Ishikawa-Ankerhold and A. Müller-Taubenberger, "Actin assembly states in *Dictyostelium discoideum* at different stages of development and during cellular stress", Int. J. Dev. Biol., Vol. 63, pp. 417-427, 2019.

[5] M. Meima and P. Schaap, "*Dictyostelium* development-socializing through cAMP", Semin. Cell. Dev. Biol., Vol. 10, pp. 567-576, 1999.

[6] R. S. Williams, K. Boeckeler, R. Gräf, A. Müller-Taubenberger, Z. Li, R. R. Isberg, D. Wessels, D. R. Soll, H. Alexander, and S. Alexander, "Towards a molecular understanding of human diseases using *Dictyostelium discoideum*", Trends. Mol. Med., Vol. 12, (9) pp. 415-24, 2006.

[7] A. Kortholt and P. J. Haastert, "Highlighting the role of Ras and Rap during *Dictyostelium* chemotaxis", Cell signal., Vol. 20, pp. 1415-1422, 2008.

[8] J. H. Raaijmakers and J. L. Bos, "Specificity in Ras and Rap signaling", J. Biol. Chem., Vol. 284, pp. 10995-10999, 2009.

[9] H. Hilbi and A. Kortholt, "Role of the small GTPase Rap1 in signal transduction, cell dynamics and bacterial infection", Small GTPases., Vol. 10, pp. 336-342, 2017.

[10] H. M. Mun, M. R. Lee, and T. J. Jeon, "RapGAP9 regulation of the morphogenesis and development in *Dictyostelium*" Biochem. Biophys. Res. Commun., Vol. 446, pp. 428-433, 2014.

[11] C. Litschko, J. Damiano-Guercio, S. Brühmann, and J. Faix, "Analysis of

Random Migration of *Dictyostelium* Amoeba in Confined and Unconfined Environments", Methods Mol. Biol., Vol. 1749, pp. 341-350, 2018.

[12] B. Park, H. Kim, and T. J. Jeon. "Loss of RapC causes defects in cytokinesis, cell migration, and multicellular development of Dictyostelium", Biochem. Biophys. Res. Commun., Vol. 499, pp. 783-789, 2018.J. H. Raaijmakers and J. L. Bos, "Specificity in Ras and Rap signaling", J. Biol. Chem., Vol. 284, pp. 10995-10999, 2009.