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Roles of RasU in Cell Motility and Development

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Abstract

Ras small GTPases are involved in regulating various cellular signaling pathways including cell migration, proliferation, and differentiation. Ras GTPase subfamily is comprised of 15 proteins; 11 Ras, 3 Rap, and one Rheb related protein. Some Ras proteins, such as RasC and RasG, have been identified for their major functions, but there are proteins whose functions have not been studied yet, such as RasU and RasX. Here, we investigated the roles of RasU in cell motility and development. RasU shows the highest homology with RasX. To investigate the functions of RasU, *rasU* null cells were used to observe the phenotype. Cells lacking RasU were larger and more spread than wild-type cells. These results indicate that RasU plays a negative role in cell spreading. In addition, we investigated the roles of RasU in cell motility and development of Dictyostelium cells and found that *rasU* null cells exhibited decreased random migration speed and delayed developmental process. These results suggest that RasU plays an important role in cell motility and development.

Keywords: Ras protein, RasU, Cell motility, Development, *Dictyostelium*

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1. Introduction

Cell migration is essential for diverse physiological processes including the recruitment of leukocytes to the sites of infection in human body, and neuronal patterning in the developments of nervous system. Misguided cell migration plays an important role in a variety of human diseases, including metastatic cancer and inflammatory diseases, such as asthma and arthritis[1].

Dictyostelium discoideum is a unicellular eukaryotic microorganism used as a model system to address many important cellular processes including cell migration, cell division, and development[2,3]. *Dictyostelium* is a free-living soil amoeba that feeds on bacteria. They chase bacteria by chemotaxing towards folic acid, which is secreted by the bacteria[4]. Upon starvation, *Dictyostelium* initiates a regulated developmental process by forming aggregates, slugs, and finally, fruiting bodies. During development single cells undergo a drastic change in gene expression and start to release the chemoattractant, cAMP, which cause neighboring cells to migrate

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in direction of increasing concentrations along the gradients to form aggregates and multicellular fruiting bodies[2,5,6].

The *Dictyostelium* Ras GTPase subfamily comprises 15 proteins; 11 Ras, 3 Rap and one Rheb related protein[4]. Ras cycles between an inactive GFP-bound and an active GTP-bound structures. Ras activation or deactivation is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively[7]. Abnormal Ras activation is associated with autism and other neurological disorders[8]. To further examine the regulatory functions of Ras, we previously undertook a bioinformatics search by *Dictyostelium* genome database and found that RasU is a Ras subfamily protein that represents high homology with RasX, which functions in cell migration and development have not yet known. Here, we studied the functions of RasU in diverse biological processed by examine phenotypes of *rasU* null cells.

2. Materials and Methods

2.1. Strains and cell culture

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

2.2. Random migration

Random motility assay was performed as described previously[9]. vegetative fully grown in 100 mm cell culture plate were resuspended. 50 μL of the cell suspension were added to 30 mm culture plate containing 3mL of Na/K phosphate buffer (pH 6.1) and allowed to adhere to the plate for 30 min. Cell migration was recorded at intervals of 1 min for 30 min using an inverted microscope (IX71; Olympus) with a camera (DS-Fil; Nikon) controlled by the NIS-Elements software (Nikon). Trajectory speed was calculated trajectory speed of cell migration every 1 min in a time-lapse recording and sequentially plotting the readings against time using Image J software (NIH).

2.3. Development assay

Development assay was performed as described previously[10]. Exponentially growing cells were washed twice with 12 mM Na/K phosphate buffer (pH 6.1) and resuspended at a density of 3.5×10^7 cells/ml and then 50 μL of the cells were dropped on Na/K phosphate agar plates and developed for 48 h. The developmental morphology of the cells was examined under a phase-contrast microscope.

3. Results

To further characterize the RasU protein, we performed computer-based analyses. *Dictyostelium* RasU (DDB_G0270138) is composed of 213 amino acids (expected molecular mass 24.7 kDa) and contains a RAS domain at the N-terminal region (Fig. 1A). We constructed phylogenetic trees with RAS proteins containing proteins. RasU showed high homology with RasX (Fig. 1B).

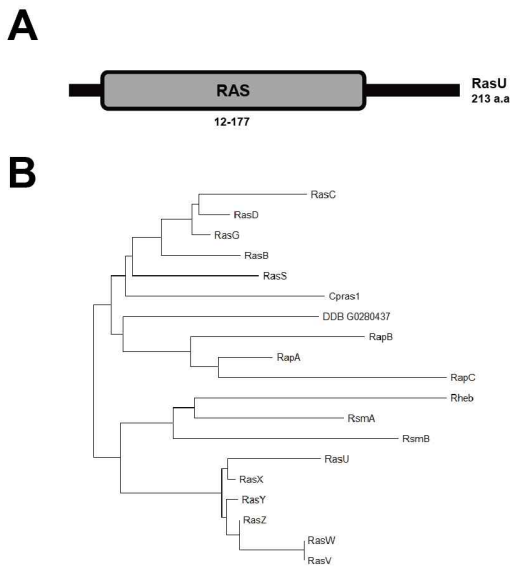

Fig. 1. Domain structure and phylogenetic tree of RasU. (A) Domain structure of RasU. RasU contains a Ras domain in *Dictyotelium* (B) Phylogenetic tree with Ras domains in *Dictyostelium*. The amino acid sequences of Ras are available at DictyBase.

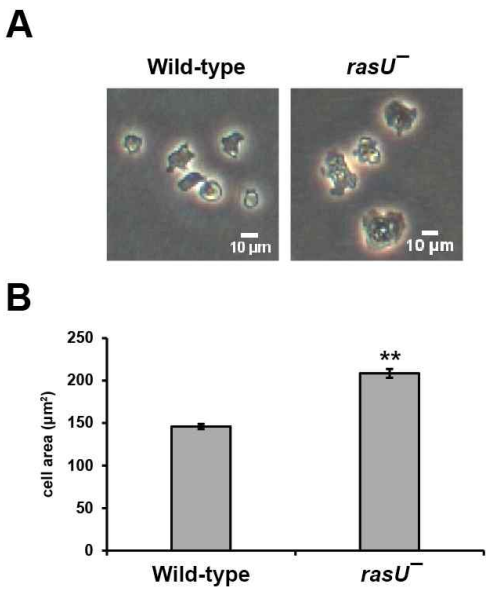

Fig. 2. RasU involved in controlling cell morphology. (A) Morphology of the vegetative cells. KAx-3 and rasU null cells were photographed. Exponentially growing cells were photographed (B) Analysis of cell area. The area of the cells was measured using Image J software. The values are the means ±SEM of three independent experiments (**p〈0.01 compared to the control by the student's *t*-test).

To investigate the function of RasU, we observed the phenotypes of wild-type and *rasU* null cells. *rasU* null cells were more spread than wild-type cells (Fig. 2A). We measured the size of the cells using NIS-element software. The mean of cell size of the *rasU* null cells was approximately 1.5-fold larger than wild-type cells (Fig. 2B).

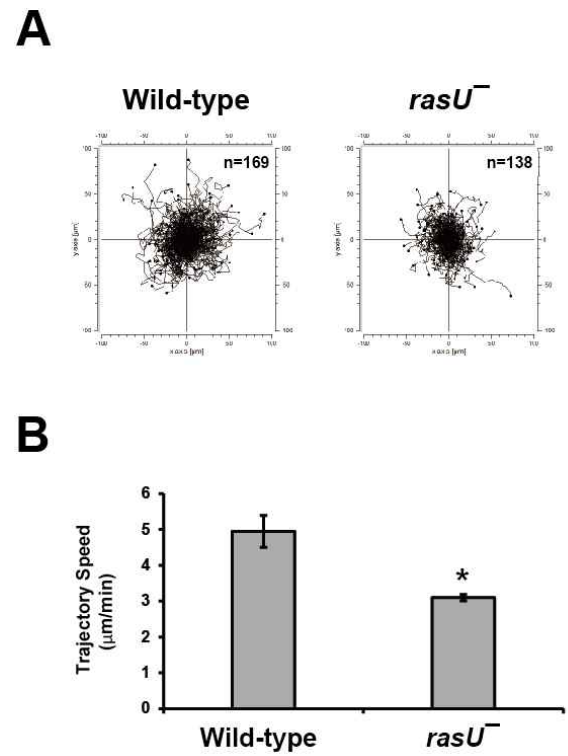

Fig. 3. Random motility of *rasU* null cells. (A) Trajectories of cells migrating randomly in 30 mm culture plate, and the movements of the cells were recorded by time lapse photography for 30 min at 1 min intervals (B) Analysis of migrating cells. The recorded images were analyzed by Image J software. Speed indicated the speed of the cell movements align the total path. Error bars represent ±SEM of three independent experiments (*p〈0.05 compared to the control by the student's *t*-test).

We examined the motility of *rasU* null cells by using Image J software and NIS-element software. In the migration trajectories of the

cells, *rasU* null cells migrated less than wild-type cells (Fig. 3A). The average speed of wild-type cells was approximately 5 μm/min and the speed of *rasU* null cells was reduced to 3 μm/min (Fig.3 B).

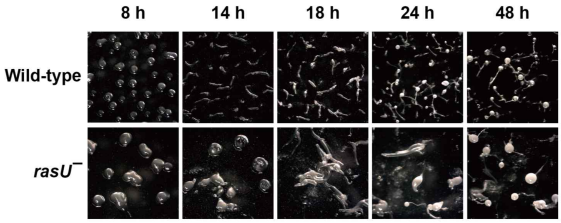

Fig. 4. Development of wild-type cells and *rasU* null cells. Development of non-nutrient agar plate. Exponentially growing cells were washed and plated on non-nutrient agar plates. photographs were taken at the indicated times after plating. Representative developmental images of the cells at 6 h (aggregation stage) and at 48 h (fruiting body formation stage) are shown.

During development, *Dictyostelium* cells release chemoattractant cAMP, initiation surrounding cells migrate, and the formation of multicellular fruiting body[2]. To examine the possible *in vivo* roles of RasU in cell motility and development, we performed development assay. As shown in Figure 4, *rasU* null cells aggregated slightly delayed forming mound at ~14 h with timing and morphology differently to those of wild-type cells. Also, the formation of fruiting body was slightly delayed in *rasU* null cells compared to wild-type cells. *rasU* null cells formed larger mounds and slugs than wild-type cells and finally formed larger fruiting bodies (Fig. 4).

4. Discussion

The small GTPase is involved in controlling diverse cellular processes, including cell adhesions, cell polarity formation, and cell migration. Eleven genes of Ras have been identified in *Dictyostelium*[2]. (RasU is a Ras subfamily protein which has not been studied yet. Present studies indicate that RasU plays a positive role in cell motility and development. *rasU* null cells showed increased cell size and were more spread compared to wild-type cells. These results suggest that RasU functions negatively in regulating cell spreading. In random migration assay, *rasU* null cells showed reduced migration speed than wild-type cells.

When depleted of nutrients, *rasU* null cells showed decreased chemotactic abilities to move toward increasing concentration and delayed forming of multicellular and developed than wild-type cells. However, *rasU* null cells formed larger mounds and slugs than wild-type cells and formed larger fruiting bodies. These results suggest that RasU is required for cell migration and development. To further confirm the functions of RasU in cell motility and development, it would be helpful to determine whether the phenotypes of *rasU* null cells are restored by overexpressing RasU proteins in the null cells. In addition, cells lacking RasU showed weak adhesion compared to wild-type cells, suggesting that RasU plays some roles in the regulation of cell adhesion.

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