Biochemical and Biophysical Research Communications 499 (2018) 783–789

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Loss of RapC causes defects in cytokinesis, cell migration, and multicellular development of *Dictyostelium*

Byeonggyu Park, Hyeseon Kim, Taeck Joong Jeon*

Department of Biology & BK21- Plus Research Team for Bioactive Control Technology, College of Natural Sciences, Chosun University, Gwangju 61452, Republic of Korea

ARTICLE INFO

Article history:
Received 25 March 2018
Accepted 30 March 2018
Available online 3 April 2018

Keywords:
RapC
Rap
Ras
Dictyostelium

ABSTRACT

The small GTPase Ras proteins are involved in diverse cellular processes. We investigated the functions of RapC, one of 15 Ras subfamily GTPases in *Dictyostelium*. Loss of RapC resulted in a spread shape of cells; severe defects in cytokinesis leading to multinucleation; decrease of migration speed in chemoattractant-mediated cell migration, likely through increased cell adhesion; and aberrations in multicellular development producing abnormal multiple tips from one mound and multi-branched developmental structures. Defects in cells lacking RapC were rescued by expressing GFP-RapC in *rapC* null cells. Our results demonstrate that RapC, despite its high sequence homology with Rap1, plays a negative role in cell spreading and cell adhesion, in contrast to Rap1, which is a key regulator of cell adhesion and cytoskeleton rearrangement. In addition, RapC appears to have a unique function in multicellular development and is involved in tip formation from mounds. This study contributes to the understanding of Ras-mediated cellular processes.

1. Introduction

Ras proteins are small monomeric GTPases that cycle between GTP-bound activated and GDP-bound inactivated forms and are involved in essential cellular processes such as cell growth and division, cell adhesion, cell migration, and vesicle trafficking [1–3]. The Ras family proteins consist of Ras (H-Ras, K-Ras, and N-Ras), Rap (Rap1A, Rap1B, Rap2A, Rap2B, and Rap2C), R-Ras, Ral, and Rheb proteins [3,4]. While Ras proteins have been extensively studied because of their roles in human oncogenesis, little is known about the other Ras-family proteins.

Rap proteins, which were initially identified as Ras antagonists, exist as several isoforms in mammals; Rap1 (Rap1A and Rap1B) and Rap2 (Rap2A, Rap2B, and Rap2C) [2,4]. Rap1 and Rap2 exhibit approximately 60% identity in their amino acid sequences. Rap1 proteins have an essential function in integrin-mediated cell substrate adhesion and cadherin-mediated cell-cell interactions, as well as phagocytosis and cell migration [2,5]. Most functions and the regulations of Rap2 are not well-understood. Generally, it is thought that Rap1 and Rap2 are regulated by a similar set of guanine exchange factors (GEF) and GTPase-activating proteins (GAP) and share most effector proteins [6]. However, some studies showed that Rap1 and Rap2 have distinct functions. It has been reported that Rap2 binds to a different set of effector proteins, Traf2 and Nck-interacting kinases (TNIKs) [6,7] and the citron homology domain of TNIK, MINK, and Map4k4, which are involved in many diverse signaling pathways.

Dictyostelium is a unicellular eukaryotic model organism that is widely used to study cellular and molecular biological processes, including the Ras signaling pathway. The *Dictyostelium* Ras GTPase subfamily contains 15 proteins, 11 Ras, 3 Rap and one Rheb-related protein [2,5]. *Dictyostelium* Rap proteins are composed of Rap1 (RapA), RapB, and RapC. Rap1 functions mainly in controlling cell adhesion through dynamic cytoskeleton rearrangements in response to diverse external stimuli, F-actin assembly and mysoin disassembly [5,8]. The functions of RapB and RapC in *Dictyostelium* have not been examined. The present study investigated the functions of RapC in cell morphology, cell adhesion, migration, and mullticellular development.

* Corresponding author. College of Natural Sciences Room 3106, Chosun University, Gwangju 61452, Republic of Korea.
E-mail address: tjeon@chosun.ac.kr (T.J. Jeon).

https://doi.org/10.1016/j.bbrc.2018.03.223
0006-291X/

2. Materials and methods

2.1. Strains and plasmid construction

Dictyostelium wild-type KAx-3 strains were grown axenically in HL5 medium or in association with *Klebsiella aerogenes* at 22 °C. Knock-out strains and transformants were maintained in 10 μg/mL blasticidin or 10 μg/mL G418. The *rapC* knockout construct was prepared by inserting the blasticidin (bsr) resistance cassette into the *BamH*I site which was created at nucleotide 535 of *rapC* gDNA and used for a gene replacement in KAx-3 parental strains. Randomly selected clones were screened for gene disruption by polymerase chain reaction (PCR), and no transcript of *rapC* in the selected *rapC* null cells was confirmed by RT-PCR. To express GFP-RapC, the full coding sequence of *rapC* was generated by PCR using cDNA extracted from wild-type cells and cloned into the *Eco*RI-*Xho*I site of the expression vector pEXP-4(+) containing a GFP fragment at the N-terminus. Expression was confirmed by immunoblotting using anti-GFP antibodies.

2.2. Cell adhesion, development and chemotaxis analysis

The cell adhesion assay was performed as described previously [9]. Development and chemotaxis analyses were performed as described previously [10]. Exponentially growing cells were harvested, washed, and then plated on Na/K phosphate agar plates at a density of 4×10^6 cells/cm^2. The developmental morphology of the cells was examined by photographing the developing cells with a phase-contrast microscope at the times indicated in the figures.

Chemotaxis toward cAMP was examined as described previously [10]. Aggregation-competent cells were prepared by incubating the vegetative cells at a density of 5×10^6 cells/mL in Na/K phosphate buffer for 10 h. Cell migration was analyzed using a Dunn chemotaxis chamber (Hawksley, Sussex, UK). Images of chemotaxing cells were acquired at time-lapse intervals of 6 s for 30 min using an inverted microscope (IX71; Olympus, Tokyo, Japan) with a camera (DS-Fil; Nikon, Tokyo, Japan). The data were analyzed using NIS-Elements software (Nikon) and ImageJ software (National Institutes of Health, Bethesda, MD, USA). The chemotaxis index (directionality) was used to quantify how directionally cells migrate towards cAMP. To assess the chemotaxis index, directionality was calculated as the shortest linear distance between the start and end points of the migration path (displacement distance) divided by the total distance traveled by a cell for 10 min. A cell moving directly towards higher gradients of cAMP would have a directionality of 1. The migration speed was evaluated by the trajectory speed, with the total distance traveled of a cell divided by time.

2.3. RT-PCR analysis

Total RNA from wild-type cells and *rapC* null cells was extracted by using the SV Total RNA Isolation system (Promega, Madison, WI, USA), and the cDNAs were synthesized by reverse transcription with MMLV reverse transcriptase (Promega) using random hexamers and 5 μg of total RNAs. A total of 5 μL cDNA was used in PCR for 35 cycles employing gene-specific primers. Universal 18S ribosomal RNA specific primers were used as an internal control [11].

2.4. Statistics

Statistical analysis was performed using Student's *t*-tests (two-tailed). All data were collected from at least three independent experiments and expressed as the means ± standard error of measurement (SEM). P value less than 0.05 was considered as statistically significant.

3. Results

3.1. RapC, the closest homolog of Rap1

Dictyostelium RapC (DDB_G0270340) is composed of 278 amino acids and contains an RAS domain at the N-terminal region (Fig. 1) and has an additional stretch of amino acids at the C-terminus, which is not found in homologs RapA and RapB. The expected molecular mass is approximately 30 kDa. The *Dictyostelium* Ras subfamily comprises of 15 proteins. Multiple alignments and phylogenetic analysis of the Ras family proteins showed that RapC is closest to Rap1 (RapA). The Ras domain of RapC showed 51% amino acid identity with that of Rap1. A threonine residue, which is critical for its functions and is known as a marker residue of Rap proteins [12], was found at amino acid residue 61, rather than glutamine, which is found in most Ras proteins.

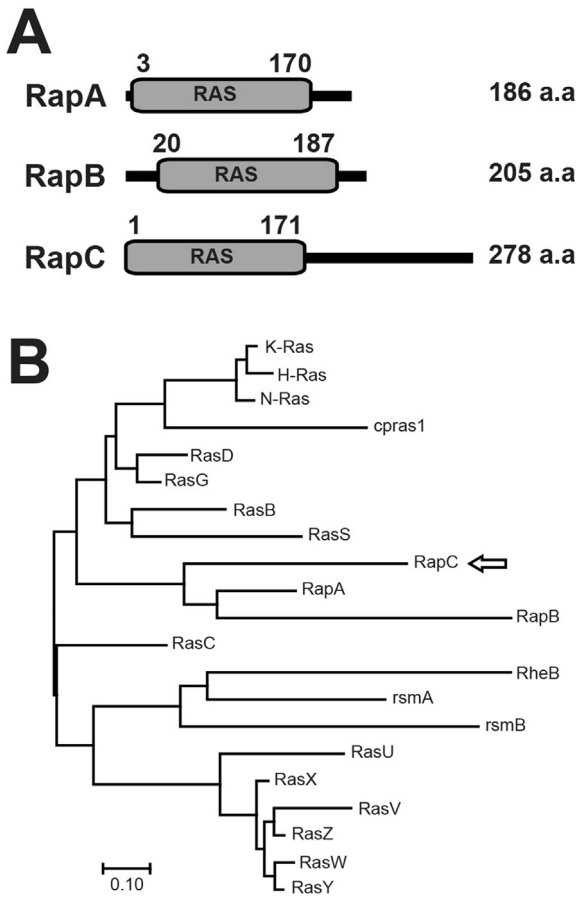

Fig. 1. RapC domain structure and phylogenetic analysis. (A) Domain structures of *Dictyostelium* Rap proteins. RapC contains an RAS domain at the N-terminus and an additional stretch of amino acids at the C-terminus. (B) Phylogenetic tree of Ras proteins. The amino acids of RAS domains were aligned by ClustalW and a phylogenetic tree was drawn to see the homology among RAS domains. *Dictyostelium discoideum* Rap1 (RapA, dictyoBaseID: G0291237); RapB (G0267456); RapC (G0270340); RasB (G0292998); RasC (G0281385); RasD (G0292996); RasG (G0293434); RasS (G0283537); RasU (G0270138); RasV (G0270736); RasW (G0270122); RasX (G0270124); RasY (G0270126); RasZ (G0270140); cpras1 (G0277381); RheB (G0277041); rsmA (G0283547); rsmB (G081253); *Homo sapiens* H-Ras (AAM12630), K-Ras (NP_203524), and N-Ras (NP_002515).