

## Loss of FrmB results in increased size of developmental structures during the multicellular development of *Dictyostelium* cells<sup>§</sup>

Hyeseon Kim, Mi-Rae Lee,  
and Taeck Joong Jeon<sup>\*</sup>

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

(Received May 30, 2017 / Revised Jul 25, 2017 / Accepted Jul 27, 2017)

**FERM domain-containing proteins are involved in diverse biological and pathological processes, including cell-substrate adhesion, cell-cell adhesion, multicellular development, and cancer metastasis. In this study, we determined the functions of FrmB, a FERM domain-containing protein, in the cell morphology, cell adhesion, and multicellular development of *Dictyostelium* cells. Our results show that FrmB appears to play an important role in regulating the size of developmental structures. *frmB* null cells showed prolonged aggregation during development, resulting in increased size of developmental structures, such as mounds and fruiting bodies, compared to those of wild-type cells, whereas FrmB over-expressing cells exhibited decreased size of developmental structures. These results suggest that FrmB may be necessary for limiting the sizes of developmental structures. Loss of FrmB also resulted in decreased cell-substrate adhesion and slightly increased cell area, suggesting that FrmB had important roles in the regulation of cell adhesion and cell morphology. These studies would contribute to our understanding of the intertwined and overlapped functions of FERM domain-containing proteins.**

**Keywords:** FrmB, FERM domain-containing protein, development, *Dictyostelium*

### Introduction

FERM domain-containing proteins are involved in diverse biological processes, including cell-substrate adhesion, cell migration, cell-cell adhesion, and multicellular development (Bretscher *et al.*, 2002; Bosanquet *et al.*, 2014). The FERM domain was named as four-point-one, ezrin, radixin, and meosin and is composed of three globular subdomains, arranged in a clover leaf structure (Bretscher *et al.*, 2002). The FERM superfamily consists of over 40 proteins con-

taining a FERM domain at the N-terminal and typically an actin-binding domain at the C-terminal ends of the proteins. These proteins bind a variety of cell membrane-associated proteins and lipids and mediate the linkage between the cell membrane and the actin cytoskeleton (Bretscher *et al.*, 2002; Bosanquet *et al.*, 2014). In mammalian cells, FERM domain proteins have been implicated to play important roles in cellular migration, and certain proteins are involved in wound healing and cancer metastasis (McClatchey, 2003; Bosanquet *et al.*, 2014). However, their functions are likely to be intertwined and overlapped, and further studies are needed to elucidate these functions.

In *Dictyostelium*, seven FERM domain-containing proteins have been identified in sequencing databases, including TalinA, TalinB, Myosin VII, MyoG, FrmA, FrmB, and FrmC (Enlazin) (Tuxworth *et al.*, 2001; Octaviani *et al.*, 2006; Patel *et al.*, 2008; Tsujioka *et al.*, 2008; Patel and Brunton, 2009; Breshears *et al.*, 2010). All FERM domain-containing proteins except FrmB have been characterized and are known to be involved in cell-substrate adhesion and motility. TalinA and TalinB are well known adaptor proteins linking the intracellular cytoskeleton and molecules on the membrane. TalinA directly interacts with Myosin VII and regulates cell-substrate adhesion (Tuxworth *et al.*, 2005). TalinB is also necessary for adhesion during *Dictyostelium* morphogenesis (Plak *et al.*, 2016). MyoG plays an important role in cellular adhesion, extension of actin-filled projections, such as filopodia and stereocilia, and directional migration. FrmA regulates the temporal and spatial control of TalinA and paxillin at cell-substratum adhesion sites, which in turn controls adhesion and motility (Patel *et al.*, 2008). FrmA plays a critical role in regulating cell-cell adhesion and multicellular development in *Dictyostelium* (Patel and Brunton, 2009). FrmC has also been shown to play a role in the regulation of cell-substrate adhesion and cytokinesis, as loss of FrmC results in reduced cell-substrate adhesion (Octaviani *et al.*, 2006).

In this study, we investigated the roles of FrmB (DDB-0233516) in *Dictyostelium*, a FERM domain-containing protein, in biological processes such as cell morphology, cell adhesion, and multicellular development by comparing the phenotypes of *frmB* null cells and FrmB-overexpressing cells. We found that FrmB was involved in regulating the size of developmental structures, such as aggregates and fruiting bodies, during the multicellular development of *Dictyostelium* cells.

<sup>\*</sup>For correspondence. E-mail: tjeon@chosun.ac.kr; Tel.: +82-62-230-6654; Fax: +82-62-230-6654

<sup>§</sup>Supplemental material for this article may be found at <http://www.springerlink.com/content/120956>.

Copyright © 2017, The Microbiological Society of Korea

## Materials and Methods

### Strains and plasmid construction

*Dictyostelium* wild-type KAX-3 cells were cultured axenically in HL5 medium or in association with *Klebsiella aerogenes* at 22°C. The knock-out strains and transformants were maintained in 10 µg/ml blasticidin and 10 µg/ml of G418. The *frmB* knockout construct was made by inserting the blasticidin resistance cassette (*bsr*) into *Bam*HI site created at nucleotide 1030 of *frmB* gDNA and used for a gene replacement in KAX-3 parental strains. Randomly selected clones were screened for a gene disruption by PCR. For expression of GFP-FrmB, the full coding sequence of *frmB* was generated by PCR and the primers which are complemented with the sequences at the beginning and the end of the *frmB* gene, cloned into the *Eco*RI-*Xho*I site of the expression vector pEXP-4(+) containing a GFP fragment. The plasmids were transformed into KAX-3 cells or *frmB* null cells, and the cells were maintained in 10 or 50 µg/ml of G418. The primers used in the screening for a gene replacement are following: Forward primers I (5'-CCC GAATTCATGGAATCATCAT TTTGAAGC-3') and II (5'-GTAATTATCCATGTGAAGAGG-3'), and Reverse primers III (5'-AAAATTTTTTTTATCTAGAGGATC-3'), IV (5'-CCCCTCGAGAAATTATTTCTTTTTTGAATC-3'), and V (5'-TTTAATACTCGTATTCC C-3').

### Cell adhesion assay

Cell adhesion assay was performed as described previously (Mun *et al.*, 2014). Log-phase growing cells on the plates were washed with 1× Na/K phosphate buffer, and then the amount of  $2 \times 10^6$  cells in 100 µl were placed on the 6 well culture dishes for overnight. The cells were photographed and counted for calculating the total cell number. To quantify attached cell number, plates were then shaken at 150 rpm for 1 h, after that the medium was removed. The number of attached cells in plates was counted (attached cells). Cell adhesion was presented as a percentage of attached cells compared with total cells.

### Development analysis

The Development was performed as described previously (Jeon *et al.*, 2009). Exponentially growing cells were harvested and washed twice with 12 mM Na/K phosphate buffer (pH 6.1) and plated on Na/K phosphate agar plates at a density of  $3.5 \times 10^7$  cells/cm<sup>2</sup>. For development of the cells under submerged conditions, exponentially growing cells were washed, and then  $2 \times 10^6$  cells were transferred into 12-well plates containing Na/K phosphate buffer. The developmental morphology of the cells was examined by photographing the developing cells with a phase-contrast microscope.

### RT-PCR analysis

The total RNAs from wild-type cells and *frmB* null cells were extracted by using the SV Total RNA Isolation System (Promega), and the cDNAs were synthesized by reverse transcription with MMLV reverse transcriptase (Promega) using random hexamers and 5 µg of total RNAs. 5 µl of the cDNAs

were used in the following PCR with 35 cycles employing gene-specific primers. The universal 18S ribosomal RNA specific primers were used as an internal control (Schroeder *et al.*, 2001; Jeon *et al.*, 2007).

## Results and Discussion

### FrmB, a FERM domain-containing protein

FERM domain-containing proteins act as mediators by linking the cytoskeleton to the membrane and are involved in cell adhesion and cell migration (Bretscher *et al.*, 2002). Recent reports have demonstrated that these proteins play important roles in cancer metastasis and development (McClatchey, 2003; Bosanquet *et al.*, 2014). To further understand the functions of FERM domain-containing proteins, we performed a bioinformatics database search using the SMART website (<http://smart.embl-heidelberg.de/>) with combinations of a specific domain (the B41 domain) and particular species (*Dictyostelium discoideum*). We identified seven FERM domain proteins, i.e., FrmA, FrmB, FrmC, TalinA, TalinB, MyoG, and MyoI (MyosinVII). Six proteins have been reported in the previous studies (Tuxworth *et al.*, 2001; Octaviani *et al.*, 2006; Patel *et al.*, 2008; Tsujioka *et al.*, 2008; Patel and Brunton, 2009; Breshears *et al.*, 2010), and MyoG was also identified as a FERM domain-containing protein. The schematic structures of FERM domain-containing proteins showed that FrmA and MyoG had two FERM domains, and all other proteins contained only one FERM domain (Supplementary data Fig. S1A). All of these proteins, except FrmB (DDB0233516), had been characterized previously. *Dictyostelium* FrmB is composed of 478 amino acids (theoretical molecular mass; 53 kDa) and contains a FERM domain (also called B41) in the center. Phylogenetic analysis using the amino acid sequences of the FERM domains showed that the FERM domain of FrmB was relatively close to those of FrmC and MyoG (Supplementary data Fig. S1B).

FrmC was identified as a genetic suppressor of the cytokinesis defects of cortexillin-I mutants and has been erroneously referred to as Enlazin (Octaviani *et al.*, 2006). This protein acts as a membrane actin-tethering protein and contributes to cell adhesion, cortical mechanics, and cytokinesis dynamics. MyoG is required for cell polarization and chemotaxis toward cAMP during development, resulting in the failure of the *myoG* null cells to polarize and move toward aggregation centers in streams (Breshears *et al.*, 2010).

### Confirmation of *frmB* knockout cells and FrmB-overexpressing cells

To further understand the roles of FrmB in *Dictyostelium*, we prepared the *frmB* null strains by homologous recombination. The *frmB* knockout construct was prepared by inserting the blasticidin resistance (*bsr*) cassette into *frmB* genomic DNA and used for gene replacement in KAX-3 parental strains (Supplementary data Fig. S2). Randomly selected clones were screened by polymerase chain reaction (PCR) with four sets of primers and genomic DNAs, which were extracted from wild-type and *frmB* null cells. PCR with II/IV, II/V, and I/IV primers produced bands of 605, 863, and 1510 bp, respectively, in wild-type cells and bands of 2010,

2268, and 2915 bp, respectively, in *frmB* null cells; the increased size was consistent with the insertion of the *bsr* cassette (approximately 1.4 kb) into the gene. When primer III, which was located inside of the *bsr* cassette, was used in PCR, a band of 636 bp was observed only in *frmB* null cells (Supplementary data Fig. S2A). These results indicate that the *frmB* gene was replaced with the *frmB* knockout construct in *frmB* null cells. Reverse transcription (RT)-PCR using the primer set I/IV and cDNA from wild-type and *frmB* null cells confirmed that the *frmB* gene was not transcribed in *frmB* null cells (Supplementary data Fig. S2C). It has been reported at dictyExpress that *frmB* gene is expressed from the vegetative stage to the slug stage of development (Rosengarten *et al.*, 2015). To examine the functions of FrmB, cells overexpressing GFP-FrmB fusion proteins (expected size: 80 kDa) were prepared, and the expression of the protein was confirmed by western blotting with anti-GFP antibodies (Supplementary data Fig. S2D). GFP-FrmB was observed in the cytosol of the cells (data not shown).

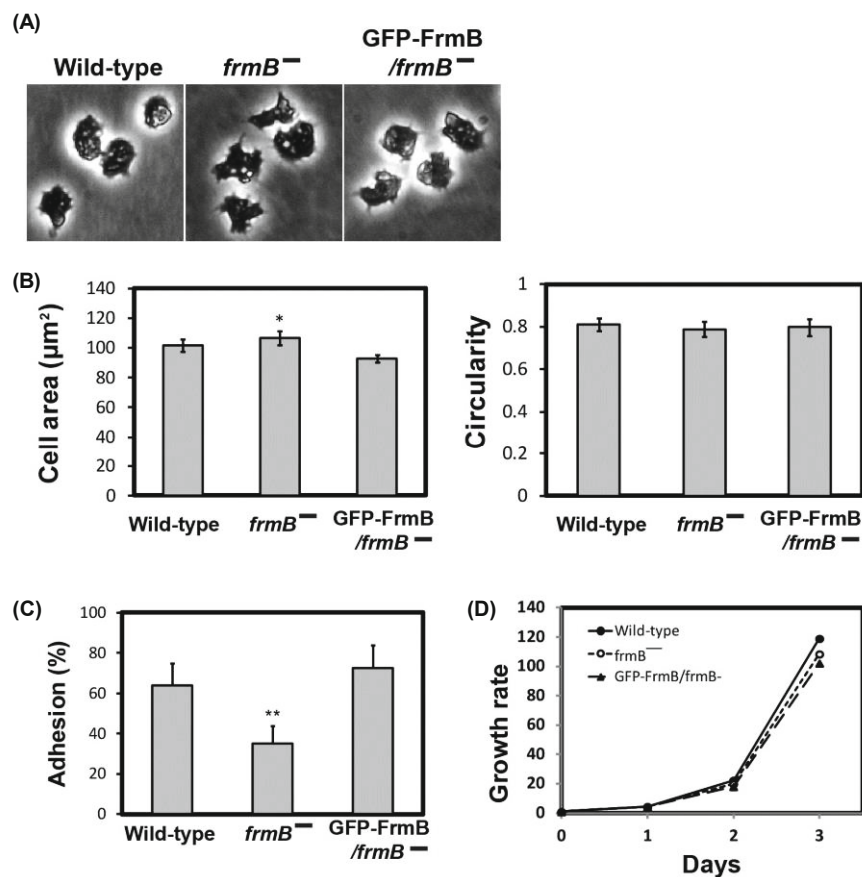
### FrmB is involved in the regulation of cell adhesion and cell morphology

To investigate the function of FrmB in cells, we examined the phenotypes of *frmB* null cells and FrmB-overexpressing cells and compared these phenotypes with those of wild-type cells. Interestingly, *frmB* null cells were slightly more spread out than wild-type cells (Fig. 1A). The phenotype of *frmB* null cells was complemented by introduction of FrmB into

the *frmB* null cells. Quantification of the cell area using NIS-Element and Image J software showed that the cell area was slightly increased in *frmB* null cells and that there was no significant difference in the roundness of the cells (circularity; Fig. 1B). The average circularity index of the cells was approximately  $0.8 \pm 0.03$ , with a circularity index of 1 representing a perfect circle.

To determine whether FrmB plays some roles in cell adhesion, the strength of cell adhesion of the *frmB* null cells was examined by shaking and washing the cell culture plate and counting the number of attached cells. From this analysis, *frmB* null cells displayed an approximate 1.7 fold decrease in cell adhesion compared to wild-type cells (Fig. 1C). This phenotype of *frmB* null cells was completely rescued by expressing FrmB in the mutant cells. Additionally, *frmB* null cells expressing FrmB showed similar or slightly stronger adhesion to wild-type cells. The growth rates of the strains were all similar (Fig. 1D). These results suggest that FrmB is required for cell-substrate adhesion and is involved in the regulation of cell morphology.

It is very interesting that FrmB seems to play a role opposite to that of FrmA in cell-substrate adhesion. Loss of FrmA leads to increased cell-substrate adhesion and overexpression of FrmA decreases cell-substrate adhesion (Patel and Brunton, 2009). In contrast, FrmC seems to play a role similar to that of FrmB in cell adhesion. Reduced expression of FrmC by RNA interference (RNAi) results in lower cell adhesion compared with that in wild-type cells (Ottaviani *et al.*, 2006).



**Fig. 1. Phenotypes of *frmB* null cells.** (A) Morphology of wild-type cell, *frmB* null cells, and *frmB* null cells expressing GFP-FrmB. Exponentially growing cells were photographed. (B) Analyses of the cell area and circularity. The area and circularity were measured and graphed. The values are the means  $\pm$  SD of three independent experiments (\* $P < 0.05$  compared to the control). (C) Cell-substrate adhesion. Adhesion of the cells was expressed as a percentage of attached cells to total cells (\*\* $P < 0.01$  compared to the control). (D) Growth rates of the cells. Wild-type cells, *frmB* null cells, and *frmB* null cells expressing GFP-FrmB cells were transferred into axenic shaking culture medium and then counted at intervals thereafter. Error bars represent SD. Statistically different from control at \* $P < 0.05$  and \*\* $P < 0.01$  by the student's *t*-test.

FrmC has also been reported to contribute to cell growth and cell spreading, as demonstrated by slow growth rates and reduced cell sizes and cell spreading in FrmC RNAi-induced cells (Octaviani *et al.*, 2006), which are different from the phenotypes of *frmB* null cells.

### Loss of FrmB results in increased size of developmental structures during multicellular development in *Dictyostelium* cells

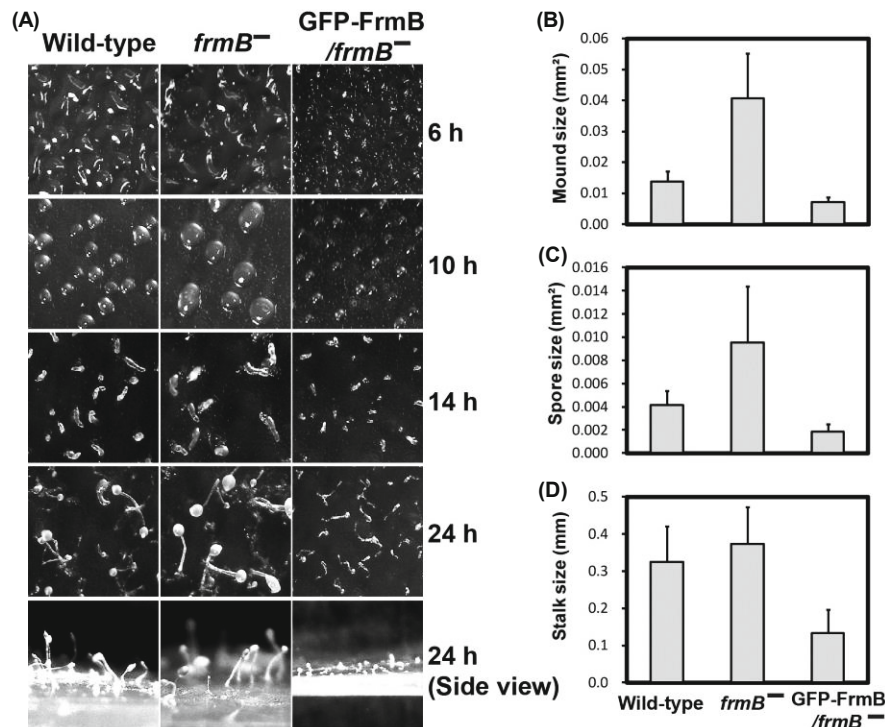
*Dictyostelium* cells live in single-cell state under nutrient-rich conditions. Depletion of nutrients from the medium triggers the developmental process over a period of 24 h. The first step of development is to form streams and mounds, which is mediated by chemotaxis of the cells to cAMP. The cells within the aggregates differentiate and sort into several types of cells, form slugs, and finally culminate and form fruiting bodies consisting of dead stalks and a mass of spores on the top (Loomis, 2015). FERM domain-containing proteins, including FrmA, MyoG, and TalinB, have been reported to be required for proper multicellular development in *Dictyostelium* cells (Tsujioka *et al.*, 2008; Patel and Brunton, 2009; Breshears *et al.*, 2010).

To determine the roles of FrmB in development, we examined the phenotypes of *frmB* null cells and FrmB-overexpressing cells in development (Fig. 2). Wild-type cells migrated into the center of the aggregates and formed mounds at 6–10 h after starvation, slugs at 14 h, and fruiting bodies within 24 h (Fig. 2A). Notably, *frmB* null cells aggregated normally to form mounds and fruiting bodies with slightly different timing and morphology from those of wild-type cells. The sizes of the mounds and fruiting bodies formed during the development of *frmB* null cells appeared to be much larger than those of wild-type cells. Quantification analysis showed that the sizes of the mounds and the spores by

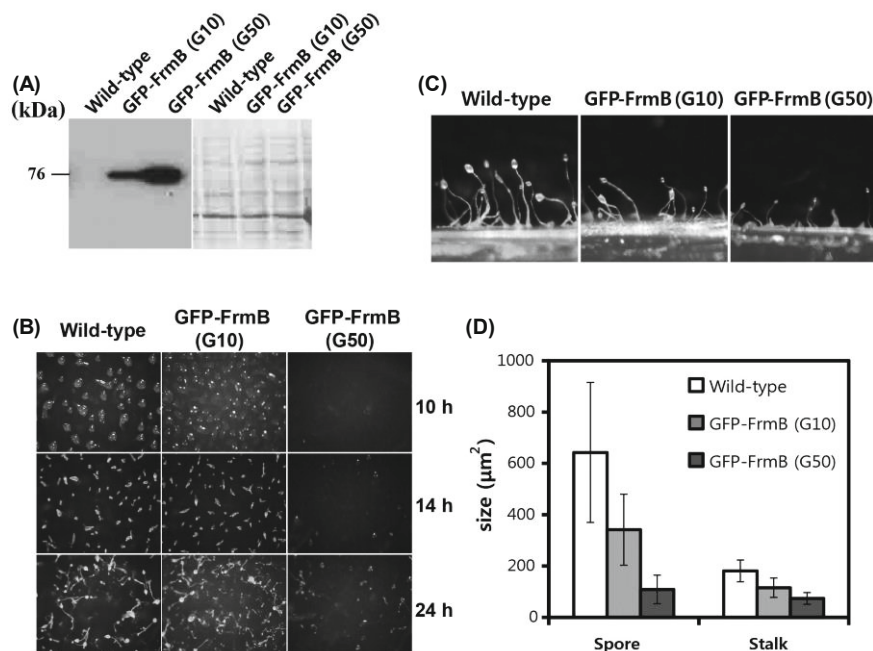
*frmB* null cells were approximately 3- (wild-type  $0.014 \pm 0.003$  mm<sup>2</sup>, *frmB* null  $0.04 \pm 0.01$  mm<sup>2</sup>) and 2.3-fold (wild-type  $0.004 \pm 0.001$  mm<sup>2</sup>, *frmB* null  $0.009 \pm 0.005$  mm<sup>2</sup>), respectively, compared to wild-type cells, and the sizes of the stalks of the fruiting bodies were similar to those of wild-type cells. The phenotypes of *frmB* null cells, increased sizes of mounds and spores, were rescued by re-expression of FrmB in the mutant cells. *frmB* Null cells expressing FrmB displayed smaller aggregates and fruiting bodies than wild-type cells. The sizes of the mounds, spores, and stalks in *frmB* null cells expressing FrmB ( $0.007 \pm 0.001$  mm<sup>2</sup>,  $0.002 \pm 0.0007$  mm<sup>2</sup>,  $0.13 \pm 0.06$  mm, respectively) were 0.5-, 0.43-, and 0.41-fold those of wild-type cells (Fig. 2).

### Overexpression of FrmB decreases the sizes of developmental structures

The effects of overexpression of FrmB on development were further examined using cells expressing different levels of FrmB. FrmB was introduced into wild-type cells and maintained in medium containing 10 or 50 µg/ml of G418 (Fig. 3). The levels of FrmB in the cells maintained in the presence of 10 (G10) or 50 µg/ml (G50) were examined by western blotting with anti-GFP antibodies; the results showed much higher levels of FrmB in the cells in G50 medium compared to G10 medium. Development of these cells showed that the sizes of the spores and stalks decreased according to the expression level of FrmB (Fig. 3). Most of the cells expressing high levels of FrmB hardly carried out developmental progression, and only a few cells formed some small-sized fruiting bodies. These results demonstrate that FrmB plays important roles in controlling the sizes of multicellular developmental structures during development. FrmB seems to be necessary for limiting the sizes of the developmental struc-



**Fig. 2.** Developmental phenotypes of *frmB* null cells. (A) Developmental phenotypes of wild-type, *frmB* null cells, and GFP-FrmB/*frmB* null cells. Exponentially growing cells were washed with Na/K buffer and plated on non-nutrient agar plates. Photographs were taken at the indicated time after plating. (B) Size of the aggregates at 10 h of development. (C) Size of the spores and stalks (D) of the fruiting bodies at 24 h.



**Fig. 3.** Developmental phenotypes of FrmB-overexpressing cells. (A) Examination of the expression level of GFP-FrmB. Proteins from cells expressing GFP-FrmB in the presence of 10 (G10) or 50  $\mu\text{g}/\text{ml}$  (G50) of G418 were subjected to western blotting with anti-GFP antibodies. (B) Development of cells expressing different levels of GFP-FrmB on non-nutrient agar plates. (C) Side view of fruiting bodies at 24 h of development. (D) Quantification of the sizes of the spores and stalks of fruiting bodies.

tures, such as mounds and fruiting bodies.

*frmA* Null cells have been reported to have defects in multicellular development, significantly delayed formation of mounds and streams, and production of short and malformed fruiting bodies compared to wild-type cells (Patel *et al.*, 2008; Patel and Brunton, 2009). The phenotypes of *frmA* null cells in development are similar to those of cells overexpressing FrmB. These results are consistent with previous suggestions that FrmA and FrmB are likely to have opposite roles in cell adhesion and development in part.

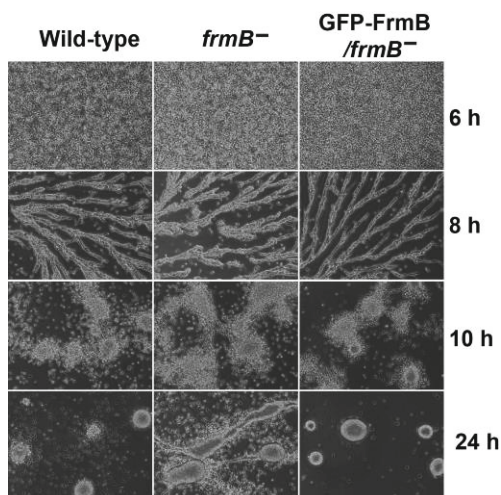
Directed cell migration along a cAMP gradient is induced by starvation of *Dictyostelium* cells to form mounds and streams

(Loomis, 2015). To determine the roles of FrmB in cell migration, we examined chemotaxis toward cAMP as a chemoattractant using a Dunn chamber. The aggregation-competent cells were prepared by incubating the cells at a density of  $5 \times 10^6$  cells/ml in Na/K phosphate buffer for 10 h (Mun *et al.*, 2014). No significant difference in chemotaxis was observed among wild-type cells, *frmB* null cells, and *frmB* null cells expressing FrmB (data not shown).

#### Prolonged aggregation of *frmB* null cells under submerged conditions

To understand the aggregation stage of development in more detail, cells were induced to develop under submerged conditions in non-nutrient Na/K buffer (Fig. 4). In these conditions, the progress of development is highly delayed and terminated at the tight mound-forming stage, enabling us to examine detailed morphological changes of multicellular structures at the mound-forming stage during development.

Wild-type cells showed streams at 8 h of development under submerged conditions, followed by the formation of loose aggregates at 10 h and tight aggregates within 24 h. *frmB* Null cells exhibited streams with timing similar to that of wild-type cells, and there was no significant morphological difference in streams compared to wild-type cells. However, at the loose or tight aggregate-forming stages (10 and 24 h, respectively), increased size of the aggregates was found in *frmB* null cells. Thus, it seems that *frmB* null cells maintain aggregation longer than wild-type cells, resulting in larger and looser aggregates. Some *frmB* null cells seemed to continue to aggregate, even after 24 h of development, and formed larger and elongated aggregates, whereas wild-type cells finished aggregation and formed tight aggregates. These phenotypes were rescued by expressing FrmB in *frmB* null cells (Fig. 4). Indeed, the streams and aggregates of *frmB* null cells expressing FrmB were thin and tight compared to wild-type or *frmB* null cells.



**Fig. 4.** Development of the cells under submerged conditions. Wild-type cells, *frmB* null cells, and *frmB* null cells expressing GFP-FrmB were washed and transferred into 12-well plates containing Na/K phosphate buffer. The images of the cells were taken at the indicated times.

These results are consistent with the previous results showing increased sizes of mounds in *frmB* null cells under normal developmental conditions on non-nutrient agar plates. Moreover, these results suggest that *frmB* null cells might have some defects in controlling the timing of stopping aggregation in the cells during development.

There are three different cell density-sensing systems in *Dictyostelium* (Gomer *et al.*, 2011). The first restricts cell proliferation during the growth phase; the second involves a signal that is secreted only when the cells starve and allows cells to sense the density of the starving cells; and the third system involves a signal that is secreted in the cells in aggregation streams and is related to the sizes of multicellular developmental structures. *Dictyostelium* cells carefully regulate the sizes of aggregates. It has been known that the third counting factor senses the number of cells in streams and leads the aggregation streams to break up, allowing the sizes of the aggregates to be constant (Brock and Gomer, 1999; Gomer, 1999). Disruption of the genes involved in this counting factor has been reported to result in small numbers of huge aggregates (Brock *et al.*, 2003; Brock *et al.*, 2006), similar to our present results. Further studies would be needed to determine if FrmB is involved in the cell counting systems by the counting factor.

A complex series of cellular interactions and cell cohesiveness are necessary for the development of ordered structures in multicellular organisms (Siu *et al.*, 2004, 2011). *Dictyostelium* cells have at least two adhesive systems. At the onset of development, *Dictyostelium* cells express the Ca<sup>2+</sup>-dependent cell-cell adhesion molecule DdCAD-1, which mediates the recruitment of individual cells into cell streams and mounds. The other adhesion mechanisms are mediated by CsA/gp80 and TgrC1/LagC/gp150 proteins, which contribute to the stability of cell-cell contacts in cell aggregates in the post-aggregation stages (Chisholm and Firtel, 2004; Siu *et al.*, 2011). The levels of these adhesion molecules are correlated with the sizes of aggregates (Gomer *et al.*, 2011; Siu *et al.*, 2011). One possibility for the prolonged aggregation and increased sizes of developmental structures in *frmB* null cells during development could be due to increased cell-cell adhesion among cells in the aggregates and remains to be tested.

In this study, we determined the roles of FrmB, a FERM domain-containing protein, in cell morphology, cell adhesion, and multicellular development of *Dictyostelium* cells by comparing the phenotypes of *frmB* null cells and GFP-FrmB over-expressing cells to those of wild-type cells. Our results suggest that FrmB might play an important role in regulating the sizes of developmental structures during the development of *Dictyostelium* cells. FrmB is also required for cell-substrate adhesion and is involved in the regulation of cell morphology. FrmB is expected to exert these functions as in other FERM domain-containing proteins, which mediate the linkage between the cell membrane and the actin cytoskeleton. Further studies would be informative to determine binding partners and if FrmB is involved in the cell counting system and cell-cell adhesion among cells in the aggregates.

## Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2016R1A1B03) and by research funds from Chosun University to T.J. Jeon (2016).

## References

- Bosanquet, D.C., Ye, L., Harding, K.G., and Jiang, W.G. 2014. FERM family proteins and their importance in cellular movements and wound healing (review). *Int. J. Mol. Med.* **34**, 3–12.
- Breshears, L.M., Wessels, D., Soll, D.R., and Titus, M.A. 2010. An unconventional myosin required for cell polarization and chemotaxis. *Proc. Natl. Acad. Sci. USA* **107**, 6918–6923.
- Bretscher, A., Edwards, K., and Fehon, R.G. 2002. ERM proteins and merlin: integrators at the cell cortex. *Nat. Rev. Mol. Cell. Biol.* **3**, 586–599.
- Brock, D.A. and Gomer, R.H. 1999. A cell-counting factor regulating structure size in *Dictyostelium*. *Genes Dev.* **13**, 1960–1969.
- Brock, D.A., Hatton, R.D., Giurgiutiu, D.V., Scott, B., Jang, W., Ammann, R., and Gomer, R.H. 2003. CF45-1, a secreted protein which participates in *Dictyostelium* group size regulation. *Eukaryot. Cell* **2**, 788–797.
- Brock, D.A., van Egmond, W.N., Shamoo, Y., Hatton, R.D., and Gomer, R.H. 2006. A 60-kilodalton protein component of the counting factor complex regulates group size in *Dictyostelium discoideum*. *Eukaryot. Cell* **5**, 1532–1538.
- Chisholm, R.L. and Firtel, R.A. 2004. Insights into morphogenesis from a simple developmental system. *Nat. Rev. Mol. Cell Biol.* **5**, 531–541.
- Gomer, R.H. 1999. Gene identification by shotgun antisense. *Methods* **18**, 311–315.
- Gomer, R.H., Jang, W., and Brazill, D. 2011. Cell density sensing and size determination. *Dev. Growth Differ.* **53**, 482–494.
- Jeon, T.J., Lee, D.J., Lee, S., Weeks, G., and Firtel, R.A. 2007. Regulation of Rap1 activity by RapGAP1 controls cell adhesion at the front of chemotaxing cells. *J. Cell Biol.* **179**, 833–843.
- Jeon, T.J., Lee, S., Weeks, G., and Firtel, R.A. 2009. Regulation of *Dictyostelium* morphogenesis by RapGAP3. *Dev. Biol.* **328**, 210–220.
- Loomis, W.F. 2015. Genetic control of morphogenesis in *Dictyostelium*. *Dev. Biol.* **402**, 146–161.
- McClatchey, A.I. 2003. Merlin and ERM proteins: unappreciated roles in cancer development? *Nat. Rev. Cancer* **3**, 877–883.
- Mun, H., Lee, M.R., and Jeon, T.J. 2014. RapGAP9 regulation of the morphogenesis and development in *Dictyostelium*. *Biochem. Biophys. Res. Commun.* **446**, 428–433.
- Octaviani, E., Effler, J.C., and Robinson, D.N. 2006. Enlazin, a natural fusion of two classes of canonical cytoskeletal proteins, contributes to cytokinesis dynamics. *Mol. Biol. Cell.* **17**, 5275–5286.
- Patel, H. and Brunton, V.G. 2009. Loss of FrmA leads to increased cell-cell adhesion and impaired multi-cellular development of *Dictyostelium* cells. *Cell Mol. Life Sci.* **66**, 145–155.
- Patel, H., Konig, I., Tsujioka, M., Frame, M.C., Anderson, K.I., and Brunton, V.G. 2008. The multi-FERM-domain-containing protein FrmA is required for turnover of paxillin-adhesion sites during cell migration of *Dictyostelium*. *J. Cell Sci.* **121**, 1159–1164.
- Plak, K., Pots, H., Van Haastert, P.J., and Kortholt, A. 2016. Direct interaction between TalinB and Rap1 is necessary for adhesion of *Dictyostelium* cells. *BMC Cell Biol.* **17**, 1.
- Rosengarten, R.D., Santhanam, B., Fuller, D., Katoh-Kurasawa, M.,

- Loomis, W.F., Zupan, B., and Shaulsky, G. 2015. Leaps and lulls in the developmental transcriptome of *Dictyostelium discoideum*. *BMC Genomics* **16**, 294.
- Schroeder, S., Kim, S.H., Cheung, W.T., Sterflinger, K., and Breuil, C. 2001. Phylogenetic relationship of *Ophiostoma piliferum* to other sapstain fungi based on the nuclear rRNA gene. *FEMS Microbiol. Lett.* **195**, 163–167.
- Siu, C.H., Harris, T.J., Wang, J., and Wong, E. 2004. Regulation of cell-cell adhesion during *Dictyostelium* development. *Semin. Cell Dev. Biol.* **15**, 633–641.
- Siu, C.H., Sriskanthadevan, S., Wang, J., Hou, L., Chen, G., Xu, X., Thomson, A., and Yang, C. 2011. Regulation of spatiotemporal expression of cell-cell adhesion molecules during development of *Dictyostelium discoideum*. *Dev. Growth Differ.* **53**, 518–527.
- Tsujioka, M., Yoshida, K., Nagasaki, A., Yonemura, S., Muller-Taubenberger, A., and Uyeda, T.Q. 2008. Overlapping functions of the two talin homologues in *Dictyostelium*. *Eukaryot. Cell* **7**, 906–916.
- Tuxworth, R.I., Stephens, S., Ryan, Z.C., and Titus, M.A. 2005. Identification of a myosin VII-talin complex. *J. Biol. Chem.* **280**, 26557–26564.
- Tuxworth, R.I., Weber, I., Wessels, D., Addicks, G.C., Soll, D.R., Gerisch, G., and Titus, M.A. 2001. A role for myosin VII in dynamic cell adhesion. *Curr. Biol.* **11**, 318–329.