 Cooperation of DEF6 with Activated Rac in Regulating Cell Morphology*

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Rho-family GTPases have been implicated in actin remodeling and subsequent morphologic changes in various cells. DEF6, a pleckstrin homology domain-containing protein, has been reported to regulate Rho-family GTPases as a guanine nucleotide exchange factor. Here, we demonstrate that DEF6 also has the property of cooperating with activated Rac1. DEF6 bound selectively to Rac1 loaded with GTP. The interaction is mediated by the effector domain of Rac1. Overexpression of GFP-DEF6 together with constitutively active Rac1 in COS-7 cells significantly changed their cell shape; this was not seen in the absence of activated Rac1. This effect of DEF6 on cellular morphology was shown to be independent of its guanine nucleotide exchange activity. Because DEF6 does not contain any sequences previously known to interact with Rac, we explored the domain necessary for the binding. The amino-terminal portion and central parts of DEF6 were required for the binding. Finally, we succeeded in creating mutants of DEF6 with point mutations in the amino-terminal portion, which abrogate the binding to activated Rac1. These mutants did not exhibit the morphologic change in COS-7 cells when they were co-expressed with active Rac1. These results suggest that DEF6 not only activates Rho-family GTPases but also cooperates with activated Rac1 to exert its cellular function.

Morphologic change of cells is mainly mediated by remodeling of the actin cytoskeleton. Rho-family GTPases, which have been suggested to be a molecular switch of signal transduction, may play critical roles in this process (1). They are active when bound to GTP and inactive when bound to GDP. Activation of GTPases is mediated by guanine nucleotide exchange factors (GEFs)3 and inactivation by their intrinsic GTPase activity, which is enhanced by GTPase-activating proteins (2, 3). After activation, they bind to specific effectors through their effector domains to activate downstream pathways (4). Among the many GEFs identified so far, Dbl-family proteins represent the major class of GEFs for Rho-family GTPases. They share a catalytic domain, called the dbl homology (DH) domain, which is followed by a pleckstrin homology (PH) domain (5, 6).

DEF6, which was originally identified as a gene in a retroviral gene-trap assay, is expressed in myeloid progenitors but down-regulated after induction of differentiation into macrophages, granulocytes, and erythrocytes in vitro (7). It is expressed at a high level in thymocytes (8). Murine DEF6 (called SLAT for Swap-70-like adapter of T cells) was shown to play a role in T-cell receptor signaling as well as in the development and maturation of Th2 cells (9). Recently, it was shown in a study with knockout mice (10) that loss of DEF6 (called IBP for IRF-4-binding protein) leads to the spontaneous development of systemic autoimmunity, suggesting a significant physiologic role of DEF6. However, the mechanisms underlying this phenotype have not been fully explored.

DEF6 contains an EF hand-like domain in the amino-terminal portion, a PH domain in the central part, and an adjacent carboxyl-terminal coiled-coil region, which is also called as DH-like region. Although the region has no statistically significant sequence homology to the DH domain, DEF6 has been shown to exhibit GEF activity for Rho-family GTPases (11, 12). However, the substrate specificity for GTPases remains elusive, and the relationship between DEF6 and GTPases has not been fully characterized.

SWAP-70 is a protein that shows a high homology to DEF6. It binds to phosphatidylinositol 3,4,5-trisphosphate (PIP3) through the PH domain and exhibits GEF activity for Rac1 (13). Kidney cells derived from mice deficient in SWAP-70 showed impaired membrane ruffling. Recently, we reported that SWAP-70 directly binds to non-muscle actin through its carboxyl-terminal region, which is missing in DEF6, and that a mutant lacking that region behaves as a dominant-negative reagent for membrane ruffling induced either by stimulation with growth factors or by expression of constitutively active Rac1 (14). Furthermore, SWAP-70 binds to activated Rac1, suggesting that SWAP-70 not only functions as a GEF but also cooperates with activated Rac1. These results prompted us to examine whether DEF6 also can bind to activated Rac1. Here we demonstrate that DEF6 interacts with activated Rac1, and we determined the region required for the binding. Point mutants incapable of binding to Rac1 lost the ability to induce a morphologic change in cell shape, which is observed when wild type DEF6 is coexpressed with constitutively active Rac1. These results suggest that DEF6 has the property of cooperating with activated Rac1 in ways other than as a GEF.
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EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—COS-7 cells and 293T cells were maintained in Dulbecco’s modified minimal essential medium supplemented with 5% fetal calf serum under a 5% CO₂ atmosphere. For electroporation of COS-7 cells, the cells were suspended in 500 µl of 30.8 mM NaCl, 120.7 mM KCl, 8.1 mM Na₂HPO₄, 1.46 mM KH₂PO₄, 5 mM MgCl₂ containing 20 µg of DNA and were pulsed by use of a CELL-PORATOR (Invitrogen) at 800 microfarads capacitance and 235 V. For GTPase activation assay, COS-7 cells were transfected with expression plasmids by FuGENE 6 reagent (Roche Applied Science) according to the manufacturer’s instruction. Introduction of DNA into 293T cells was done by the calcium phosphate precipitation method (15).

Plasmids—DEF6 cDNA was obtained by reverse transcription-PCR of total RNA isolated from Jurkat cells by the acid guanidinium thiocyanate phenol-chloroform extraction method (12) with 5'-aaagtttctggcctgcgaaggaactgctcaag-3' used as a sense primer and 5'-aagtttctggcctgcgaaggaactgctcaag-3' as an antisense primer. cDNA was subcloned into the BglII-EcoRI sites of the pEGFP-C1 vector (Clontech). Point mutations were introduced by the method of Sawano and Miyawaki (16). For deletion mutants, cDNAs encoding different segments were inserted into pEGFP-C1. pRFP-Rac1 Q61L was produced by insertion of the coding sequences of human Rac1 with a substitution of glutamine to leucine at amino acid 61 (kind gifts from Dr. M. Matsuoka) into pRFP, which was generated by substitution of the coding sequence of GFP of pEGFP-C1 with that of monomer RFP. pRFP-Cdc42 Asn-17 were generated by insertion of the coding sequences of human Cdc42 with a substitution of threonine to asparagine at amino acid 17 (kind gifts from Dr. K. Kaibuchi) into pRFP. Dominant negative human RhoA, with a substitution of threonine to asparagine at the amino acid 19 was generated by introducing point mutations into human RhoA Val-14, which was provided by Dr. K. Kaibuchi, and introduced into pRFP to generate prFP-RhoA Asn-19. Expression of RFP-Cdc42 Asn-17 and RFP-Rho Asn-19 was confirmed by Western blotting with anti-Cdc42 antibody (BD Biosciences) or anti-Rho antibody (Santa Cruz).

An expression vector for human DOCK180 was kindly provided by Dr. M. Matsuda. His-DEF6 was constructed in the pQE-30 vector (Qiagen).

Recombinant Protein Preparation—The Rac1/GTPases were subcloned into the pGEX4T3 vector to produce the expression vectors and were expressed in Escherichia coli cells, BL21. Glutathione S-transferase (GST), GST-Rac1, GST-Rho, and GST-Cdc42 recombinant proteins were purified through glutathione-Sepharose. Briefly, after induction of the proteins with 0.1 mM isopropyl-thio-β-galactopyranoside for 3 h, cells were harvested and sonicated in a lysis buffer containing 20 mM Tris-HCl (pH 7.5), 5% glycerol, 50 mM NaCl, 0.1 mM EDTA, 40 µg/ml bovine serum albumin, 4.7 mM MgCl₂, and 0.16 mM MgCl₂ for 20 min at 30 °C. Then, lysates were centrifuged for 10 min at 16,000 g at 4 °C. For a positive control, lysates were incubated with 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1% Nonidet P-40, 10 mM MgCl₂, 10% glycerol for 30 min at 4 °C. Beads were washed, and proteins bound to the resin were analyzed by Western blotting with anti-GFP or anti-DEF6 antibody.

Rac1/Cdc42 Activation Assay—Measurement of Rac1/Cdc42 activity was performed as described previously (18). Briefly, COS-7 cells transfected with various expression vectors were lysed with an ice-cold cell lysis buffer (50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 200 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride). Cell lysates were then centrifuged for 10 min at 16,000 x g at 4 °C. For a positive control lysate was incubated with 20 mM EDTA and 100 µM GTPγS at 30 °C for 10 min to load GTases in the cell lysates with GTPγS. The supernatants were mixed with GST-PAD1 PBD (p21 Rac/Cdc42-binding domain) fusion protein immobilized on glutathione-Sepharose beads for 30 min at 4 °C. The beads were washed with the lysis buffer, and the bound proteins were analyzed by SDS-PAGE and Western blotting with anti-Rac1 antibody (BD Biosciences) or anti-Cdc42 antibody (BD Biosciences). Expression of GFP fusion proteins and Myc DOCK 180 were verified by Western blotting with anti-GFP.
antibody (Santa Cruz) or anti-c-Myc antibody (Santa Cruz), respectively.

RESULTS

DEF6 Is a PIP3-binding Protein—DEF6 has a PH domain highly homologous to that of SWAP-70, which is capable of binding to PIP3 (Fig. 1A). For testing whether DEF6 also binds to PIP3, DEF6 fused with GFP was expressed in 293T cells, and its binding to PIP3-analogue beads was examined. As shown in Fig. 1B, DEF6 bound significantly to the beads. The binding was blocked by preincubation of the protein with free PIP3, suggesting that the binding was dependent on PIP3. It has been reported that several amino acids in the β1/β2 loop of the PH domain are critical for binding to PIP3 (19, 20). To explore whether the interaction of DEF6 to PIP3 is mediated by this region, we introduced mutations into the basic amino acid residues within the region to create DEF6 (K225A/R226A) and DEF6 (R230A/R231A) (Fig. 1A). These mutants were expressed as GFP fusion proteins and were examined for their PIP3 binding. As shown in Fig. 1C, these mutations abolished the activity.

DEF6 Binds to Rac1 Bound to GTP—SWAP-70 selectively binds to Rac1 bound to GTP. We tested this with DEF6. We performed pull-down assays using GST-Rac1 loaded with GTPγS or with GDP or using GST-Rac1 free of guanine nucleotides on cell lysates of 293T cells that transiently express GFP-DEF6. As shown in Fig. 2A, DEF6 bound to GST-Rac1-GTPγS but not to GST-Rac1-GDP or GST-Rac1 free of guanine nucleotides. DEF6 did not bind to GST-Cdc42 or GST-RhoA even if they were bound to GTPγS. To exclude the possibility that the interaction is indirect, we tested purified recombinant protein, His-DEF6, in an experiment similar to that illustrated in Fig. 2A (Fig. 2B). His-DEF6 bound to the GTP form of Rac1 specifically, suggesting that the interaction is direct.

![RESULTS](image1)

![RESULTS](image2)
Figure 3. Overexpression of GFP-DEF6 with RFP-Rac1QL induces morphologic change in COS-7 cells in a GEF activity independent manner. A, COS-7 cells expressing the indicated proteins are shown. Cells expressing GFP fusion proteins are shown in the middle panel, and RFP-Rac1 QL is shown in the bottom panel. Bar, 20 μm. B, COS-7 cells were transfected with expression vectors as indicated. The lysates were subjected to the CRIB pulldown assay followed by Western blotting with an anti-Cdc42 antibody (left panel). Then the antibody was stripped and reprobed with an anti-Rac1 antibody (right panel). Expression of Myc DOCK180 and GFP fusion proteins was confirmed by Western blotting with anti-myc antibody or anti-GFP antibody. In the case of the GFP-DEF6/GFP-Rac1 QL-expressing cell lysate (left panel, last lane), GTPases in the cell lysates were loaded with GTPγS to confirm that CRIB beads could afford the binding to the activated Cdc42, even the beads may be partly occupied by GFP-Rac1 QL. C, RFP, RFP-Cdc42 Asn-17, or RFP-Rho Asn-19 was expressed in COS-7 cells together with GFP-Rac1 QL and GFP-DEF6 (left panel). Expression of GFP-Cdc42 Asn-17 and RFP-Rho Asn-19 in COS-7 cells was confirmed by Western blotting with anti-Cdc42 antibody or anti-Rho antibody. Bar, 20 μm.
action of wild type DEF6 and activated Rac1 may be transient, resulting in failure of detection of the co-localization signal. DEF6-(1–407), the smallest mutant capable of binding to Rac1, caused morphologic change in COS-7 cells, as did wild type DEF6. In contrast, cells expressing DEF6-(314–631) containing DH-like region alone, which has been shown to have a GEF activity (11) but did not bind to activated Rac1 (Fig. 5B), did not exhibit the morphologic change but exhibited the membrane ruffling as is the case with the cells expressing Rac1 Q61L alone. In the case of cells expressing DEF6-(1–550), which showed stronger affinity to Rac1 compared with WT or DEF6-(1–407), the appearance was somewhat different from those of the cells expressing WT or DEF6-(1–407). Most exhibited the cell shape as shown by an arrowhead (Fig. 5C) in phase contrast image of 1–550, and some exhibited a similar shape as WT-expressing cells (arrow in phase contrast image of 1–550). Interestingly, dense signals of DEF6-(1–550) and Rac1 Q61L were localized very similarly in these cells (arrowheads in images of GFP and RFP). This may reflect the strong affinity of the DEF6 mutant to Rac1 Q61L. The different effect of this mutant on cell morphology might be explained by the strong affinity; however, the precise mechanism remains to be elucidated. Given the fact that DEF6-(1–407) lacking most part of DH-like region retained the ability to cause morphologic change, it is likely that the DH-like region for the GEF activity is not required for the morphologic change.

Some Rac-interacting proteins without any known consensus sequences have been reported (26–30). For determining what amino acids are required for the binding to Rac1 in DEF6, the amino acid sequence of DEF6 was aligned with those of the binding regions of other Rac-binding proteins. The central part of DEF6, which is required for the binding to Rac1, did not show any homology to the Rac-interacting proteins. However, amino-terminal region of DEF6 appeared to contain a stretch of amino acids weakly homologous to the Rac binding domain of these proteins (Fig. 6A). Point mutations were introduced in the conserved amino acids within DEF6 to produce R4A, L18N, and L31N/V33S (asterisks in Fig. 6A). GFP fusion proteins of these mutants were expressed in 293T cells, and the lysate was analyzed by pulldown assay with GST Rac1. As expected, the binding activity to GST-Rac1 loaded with GTP was lost in L18N and L31N/V33S, whereas weak activity remained in R4A (Fig. 6B and C).

The Mutant Incapable of Binding to Activated Rac1 Did Not Cause Morphologic Change—To see the significance of the binding of DEF6 to activated Rac1 on its cellular function, point mutants L18N and L31N/V33S fused with GFP were co-expressed with RFP-Rac1 Q61L. The cells exhibited membrane ruffling instead of the drastic morphologic change seen in cells expressing GFP-DEF6 with RFP-Rac1 Q61L (Fig. 7). Because the RFP-Rac1 Q61L expression alone results in membrane ruffling, it is likely that the point mutants are not able to induce a morphologic change and that only the effect of RFP-Rac1 Q61L was expressed by the cells. These results suggest that morphologic change of the cells induced by coexpression of DEF6 and constitutively active Rac1 is dependent on their direct interaction.

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**FIGURE 4. The effector region of Rac1 is required for the binding of DEF6 to activated Rac1.** Binding of DEF6 to various effector domain mutants of Rac1 was examined. GFP-DEF6 was pulled down with wild type or mutant GST-Rac1 immobilized on glutathione-Sepharose beads. Bound DEF6 was analyzed by Western blotting with anti-GFP antibody (top panel). The filter was stained with Coomassie Blue for estimating the amounts of GST fusion proteins present in each lane (bottom panel).

- **A** The mutant incapable of binding to activated Rac1 did not cause morphologic change in COS-7 cells, as did wild type DEF6. In contrast, cells expressing DEF6-(314–631) containing DH-like region alone, which has been shown to have a GEF activity (11) but did not bind to activated Rac1 (Fig. 5B), did not exhibit the morphologic change but exhibited the membrane ruffling as is the case with the cells expressing Rac1 Q61L alone. In the case of cells expressing DEF6-(1–550), which showed stronger affinity to Rac1 compared with WT or DEF6-(1–407), the appearance was somewhat different from those of the cells expressing WT or DEF6-(1–407). Most exhibited the cell shape as shown by an arrowhead (Fig. 5C) in phase contrast image of 1–550, and some exhibited a similar shape as WT-expressing cells (arrow in phase contrast image of 1–550). Interestingly, dense signals of DEF6-(1–550) and Rac1 Q61L were localized very similarly in these cells (arrowheads in images of GFP and RFP). This may reflect the strong affinity of the DEF6 mutant to Rac1 Q61L. The different effect of this mutant on cell morphology might be explained by the strong affinity; however, the precise mechanism remains to be elucidated. Given the fact that DEF6-(1–407) lacking most part of DH-like region retained the ability to cause morphologic change, it is likely that the DH-like region for the GEF activity is not required for the morphologic change.

- **B** Some Rac-interacting proteins without any known consensus sequences have been reported (26–30). For determining what amino acids are required for the binding to Rac1 in DEF6, the amino acid sequence of DEF6 was aligned with those of the binding regions of other Rac-binding proteins. The central part of DEF6, which is required for the binding to Rac1, did not show any homology to the Rac-interacting proteins. However, amino-terminal region of DEF6 appeared to contain a stretch of amino acids weakly homologous to the Rac binding domain of these proteins (Fig. 6A). Point mutations were introduced in the conserved amino acids within DEF6 to produce R4A, L18N, and L31N/V33S (asterisks in Fig. 6A). GFP fusion proteins of these mutants were expressed in 293T cells, and the lysate was analyzed by pulldown assay with GST Rac1. As expected, the binding activity to GST-Rac1 loaded with GTP was lost in L18N and L31N/V33S, whereas weak activity remained in R4A (Fig. 6B and C).

- **C** The mutant incapable of binding to activated Rac1 did not cause morphologic change—To see the significance of the binding of DEF6 to activated Rac1 on its cellular function, point mutants L18N and L31N/V33S fused with GFP were co-expressed with RFP-Rac1 Q61L. The cells exhibited membrane ruffling instead of the drastic morphologic change seen in cells expressing GFP-DEF6 with RFP-Rac1 Q61L (Fig. 7). Because the RFP-Rac1 Q61L expression alone results in membrane ruffling, it is likely that the point mutants are not able to induce a morphologic change and that only the effect of RFP-Rac1 Q61L was expressed by the cells. These results suggest that morphologic change of the cells induced by coexpression of DEF6 and constitutively active Rac1 is dependent on their direct interaction.
DISCUSSION

DEF6 has been reported to be an upstream activator of Rho-family GTPases (11, 12). Whereas in vitro GEF activity for Rac1 and Cdc42 but not for Rho was shown (11), DEF6 was implicated in activating all three of the members of GTPases, judging from observation of the cells overexpressing a truncated mutant of DEF6 (12). However, a precise examination of nucleotide preference in the binding of DEF6 to Rac1 has not been carried out. In this study we performed a pulldown assay with GTP-loaded Rac1, GDP-loaded Rac1, or with Rac1 free of nucleotide. DEF6 selectively binds to the activated Rac1 loaded with GTP. Our results suggest that DEF6 cooperates with activated Rac1 to exert its cellular function in some cases. Supporting this idea, overexpression of GFP-DEF6 together with activated Rac1 induced profound changes in cell morphology, which is not observed in the absence of activated Rac1. Several lines of evidence suggest that this effect is likely to be independent of the GEF activity of DEF6. Activation of Cdc42 was not detected in COS-7 cells that exhibit morphologic change and dominant negative Cdc42, or Rho did not suppress the effect. DEF6 mutants lacking most of the DH-like domain still induced the morphologic change as long as the protein maintained the Rac1 binding activity. Finally, we confirmed that the direct interaction of DEF6 and activated Rac1 was required for this morphologic change by using mutant DEF6s harboring point mutations, which abrogated the ability to bind to Rac1.

Many proteins have been reported to interact with activated Rac1 (4, 31). Whereas most of these proteins contain a conserved motif for the binding to Rac1 such as a CRIB domain (32), several proteins do not have any sequences showing homology with each other. In our search for amino acids critical for the binding to Rac1 in DEF6, we found that several amino acids were conserved among the Rac-interacting proteins. Because the introduction of a mutation into these positions abrogated the binding activity at least to some extent in DEF6, these conserved amino acids can be required for binding in the case of the other Rac-interacting proteins. Further exploration will be needed for testing this idea. On the other hand, the effector domain of Rac1 may be required for the binding to DEF6 because F37A and Y40C of Rac1, among the several effector-domain mutants, failed to bind to DEF6. Previous reports showed that several known effector proteins for Rac failed to bind to Rac carrying the mutations F37A or Y40C. For example, Rac1 F37A did not bind to p160Rock, and Rac1 Y40C did not bind to PAK (23, 33). In our previous study, SWAP-70 failed to bind to Rac1 F37A or Rac1 Y40C, which was also the case for DEF6 (14). These results suggest the possibility that these proteins use...
the same region of Rac1 as the binding site. One would argue that the morphologic change in the cells by the combination of activated Rac1 and DEF6 is the result of Rac signaling without the signals produced by the proteins that bind to Phe-37 or Tyr-40 due to blockage by the overexpressed DEF6. However, the finding that Rac1 Q61L/F37A or Rac1 Q61L/Y40C, which would mediate such unbalanced signaling, did not induce the morphologic change (data not shown) argues against this hypothesis. Therefore, it is likely that DEF6 creates some positive signals that induce the morphologic change.

DEF6 exhibits 45% sequence homology with SWAP-70. In particular, the PH domain is highly homologous to that of SWAP-70, suggesting that they have a similar function. Indeed, DEF6 also binds to PIP₃ in vitro (Fig. 1). The binding activity of the PH domain of DEF6 to PIP₃ has been examined previously (11). The investigators described that DEF6 binds to PIP₃ only when it is phosphorylated. In our study DEF6 expressed in 293T cells showed PIP₃ binding activity which they failed to detect. The difference might be caused by the sensitivity of the binding assay with phosphoinositide beads. It is possible that phosphorylated DEF6 binds to PIP₃ even more strongly than does the unphosphorylated DEF6 in our assay.

SWAP-70 has also been shown to bind to activated Rac1. However, the phenotype of the cells expressing DEF6 together with constitutively active Rac1 was different from that of the cells expressing SWAP-70 and the activated Rac1. In SWAP-70/Rac1 Q61L-expressing cells, membrane ruffling was induced, as in the case of Rac1 Q61L-expressing cells. On the other hand, DEF6/Rac1 Q61L-expressing cells exhibited a prominent morphologic change. As described above, the mechanism underlying this change is likely to be independent of the GEF activity. According to

FIGURE 6. Point mutations in the amino-terminal region of DEF6 abolished the binding activity to Rac1. A, sequence alignment of Rac-binding proteins without consensus binding motif. Identical amino acids are highlighted in black, and homologous amino acids are in gray. Positions where the point mutations were introduced are shown (asterisks). B, Rac1 binding assay was performed as described above, with use of lysates of 293T cells expressing DEF6, R4A, L18N, and L31N/V33S as GFP fusion proteins. C, the same assay as in B was performed with the indicated amounts of GST Rac1.

FIGURE 7. Mutant DEF6 with point mutations, which abolish the binding activity to Rac1, did not induce morphologic change in COS-7 cells expressing activated Rac1. GFP (control), GFP-DEF6 WT, L18N, and L31N/V33S were coexpressed with RFP-Rac1 QL. 24 h after transfection, the cells were observed under a fluorescence microscope. Bar, 20 µm.
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the previous report, wild type DEF6 exhibited the GEF activity only when it was tyrosine-phosphorylated (11). We found that DEF6 is not phosphorylated in our experimental conditions (data not shown). Therefore, DEF6 may not be active as a GEF. It is not surprising that GEF activity is not involved in the morphologic change. It is not unusual that a GEF functions in a GEF activity-independent manner. For example, Vav1, a member of Dbl family GEF, has been reported to be involved in transcriptional activation (34) or cell spreading (35) in a GEF activity-independent manner, although the mechanism of how it functions remained open.

One possible mechanism explaining the effect of DEF6 on cell shape change is that it mediates this change by acting directly to cytoskeletal proteins. Whereas SWAP-70 has the F-actin binding domain in its carboxyl-terminal region and exhibits binding activity specific for non-muscle actin, DEF6 does not have corresponding sequences. However, it has been reported that DEF6 has F-actin binding activity (12), which does not seem to be specific for non-muscle actin. The different F-actin binding property might contribute to these differential effects of constitutively active Rac1-expressing cells. Future exploration will be needed for substantiating this hypothesis.

Recently, DEF6 has been shown to play a role in the prevention of systemic autoimmunity (10). Mice deficient in DEF6 developed autoimmunity and lupus-like disease. Given that the phenotypes characteristic for the disease observed in these mice are divergent, it is unlikely that DEF6 has a sole function as a GEF. To understand the mechanism underlying the development of the disease, it would be helpful to further investigate the novel aspect of DEF6 cooperating with activated Rac.

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