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Cdc42 GTPase-activating protein (CdGAP) interacts with the SH3D domain of Intersectin through a novel basic-rich motif

Martin Primeau, Ali Ben Djoudi Ouadda, Nathalie Lamarche-Vane *

Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada H3A 2B2

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ABSTRACT

The small GTPases Rac1 and Cdc42 are key regulators of the cytoskeleton. We have previously identified the endocytic protein Intersectin as a binding partner and regulator of Cdc42 GTPase-activating protein (CdGAP) with activity towards Rac1 and Cdc42. This interaction is mediated through the SH3D domain of Intersectin and the central domain of CdGAP, which does not contain any typical proline-rich domain or known SH3-binding motif. Here, we have characterized the Intersectin-SH3D/CdGAP interaction. We show that Intersectin-SH3D interacts directly with a small region of CdGAP highly enriched in basic residues and comprising a novel conserved xKx(K/R)K motif.

Structured summary of protein interactions:
Intersectin physically interacts with **cdGAP** by pull down (View interaction)
cdGAP binds to **Intersectin** by peptide array (View interaction)
Intersectin binds to **cdGAP** by pull down (View interaction)
Intersectin physically interacts with **ARHGAP30** by pull down (View interaction).

1. Introduction

The Rho family of small GTPases, including Cdc42, Rac1, and RhoA, controls a wide variety of cellular processes ranging from cell proliferation, polarization, motility, and adhesion to intracellular membrane trafficking [1]. These proteins function as molecular switches that cycle between an active GTP-bound and an inactive GDP-bound form. Three families of regulators tightly regulate this cycle. Guanine nucleotide exchange factors (GEFs) activate GTPases by inducing the exchange of GDP for GTP, whereas guanine nucleotide dissociation inhibitors sequester and maintain inactive Rho GTPases in the cytoplasm [2]. GTPase-activating proteins (GAPs) increase the intrinsic GTPase activity, leading to inactivation of Rho GTPases [2,3].

CdGAP (Cdc42 GTPase-activating protein) promotes the inactivation of Rac1 and Cdc42 but not RhoA [4]. It comprises a N-terminal GAP domain, a basic-rich (BR) central region, and a proline-rich domain (PRD) with an extended C-terminal domain [20]. We have previously identified CdGAP as a binding partner of the endocytic scaffolding protein Intersectin involved in clathrin-mediated endocytosis and cell signalling [5]. Intersectin is composed of two N-terminal Eps-homology (EH) domains, a putative coil-coiled domain and five C-terminal SH3 domains (SH3 A–E) [6]. Its neuronal splice-variant, Intersectin-1L, contains an additional GEF domain active towards Cdc42, followed by a C2 domain [7]. Intersectin is targeted to clathrin-coated pits through the interaction of its EH domains with epsin [8]. Additionally, it can bind to various endocytic and signalling proteins, including dynamin, synaptojanin, Sos1, Numb, Wiskott-Aldrich syndrome protein (WASP), and CdGAP through a subset of its SH3 domains [7–12]. Interestingly, we have previously found that the SH3D of Intersectin negatively regulates the GAP activity of CdGAP in vitro [5]. Although CdGAP-PRD is required for the regulation of its GAP activity by Intersectin, this domain does not mediate CdGAP-Intersectin-SH3D interaction. In fact, Intersectin-SH3D interacts with the central region, lacking the conventional class I ([R/K]xXPxXP) or class II (XPxXPx[R/K]) SH3 binding motifs or other known SH3 binding motifs [13–18]. Therefore, we sought to determine which residues of CdGAP are responsible for the interaction with Intersectin-SH3D.

In this study, we demonstrate that Intersectin-SH3D directly associates with the BR region of CdGAP through a novel conserved SKSKK motif. This BR region is evolutionary conserved within CdGAP closest homologs and we show that the CdGAP-related protein ARHGAP30 is also able to interact with Intersectin-SH3D. Furthermore, the SKSKK motif is essential to CdGAP activity in vitro.

* Corresponding author. Address: 3640 University Street, Montreal, QC, Canada H3A 2B2. Fax: +1 514 398 5047.
E-mail address: nathalie.lamarche@mcgill.ca (N. Lamarche-Vane).

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2. Materials and methods

2.1. Plasmids

CdGAP constructs and pGEX4T1-actopaxin (provided by Dr. Chris Turner, SUNY, Syracuse, NY) were described elsewhere [5,19,20]. CdGAP deletion mutants were introduced into BamHI/XbaI of pRK5myc. For S^{35}-methionine in vitro translation, CdGAP-(1–515) was subcloned into BamHI/XbaI of pCDNA3.1. The SH3D domain of mouse Intersectin (a.a. 1070–1131) was subcloned into BamHI/NotI of pGEX-4T3. ARHGAP30 and ARHGAP30 deletion mutants were amplified by polymerase chain reaction (PCR) using mouse ARHGAP30 cDNA (MGC:99989, Mammalian Gene Collection, NIH) and subcloned into BamHI/XbaI of pRK5myc. CdGAP point mutants were generated by a two-step overlap extension PCR strategy using pRK5myc-CdGAP-(1–515) as a template and according to standard protocols. The PCR fragments were subcloned into BamHI/XbaI of pRK5myc or into Cla1 of pEGFPC1-CdGAP (1–820). All plasmids were verified by sequencing.

2.2. Cell culture and transfection

HEK293 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and antibiotics and maintained in a 5% CO_2 humidified environment at 37 °C. Cells were transfected with linear polyethylenimine (PEI) MW 25 000 (Polysciences) using a 1:5 ratio (DNA: PEI) or by calcium phosphate [21].

2.3. GST pull-down assay

GST, GST-SH3D, and GST-actopaxin proteins were purified as previously described [5,19]. HEK293 cells transfected with the various plasmids were lysed in 20 mM HEPES pH 7.4 and 1% Triton X-100 supplemented with 5 mM Na_3VO_4, 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF) and Complete™ protease inhibitors. After a 10-min centrifugation, supernatants were incubated with 0.6 nmol of GST or GST-SH3D and glutathione–agarose beads for 2 h at 4 °C. Samples were washed three times in lysis buffer and bound proteins were submitted to SDS–PAGE and Western blot analysis using anti-myc antibodies.

2.4. In vitro ^{35}S-methionine translation

In vitro transcription and translation were performed using TNT® Quick Coupled Transcription/Translation Systems (Promega). Briefly, TNT Quick Master mix was incubated with 1 μg of pCDNA-CdGAP(1–515) and 63 μCi of [^{35}S]-methionine in a total volume of 50 μL at 30 °C for 90 min. Fractions of the reaction mix (15 μl) were incubated with 20 μg of GST, GST-SH3D or GST-actopaxin coupled to glutathione–agarose beads, and GST pull-down assays were performed as described above. Bound proteins were submitted to SDS–PAGE, transferred to nitrocellulose membrane, and stained with Coomassie Blue. ^{35}S-methionine labelled proteins were detected by PhosphoImager analysis (Perkin–Elmer).

2.5. Peptide overlay assay

A total of 20 overlapping 15-mer peptides corresponding to CdGAP (249–358) were synthesized directly on a cellulose membrane from their C-terminus (JPT Peptide Technologies GmbH, Berlin, Germany). The membrane was washed once in 100% ethanol and three times in TBS buffer (50 mM Tris–HCl pH 8.0, 137 mM NaCl and 2.7 mM KCl) before incubation in blocking buffer (BSA 2% w/v in TBS buffer). The membrane was incubated overnight at 4 °C with GST or GST-SH3D (1.4 μmol/ml) in blocking buffer, washed in T-TBS buffer (TBS buffer supplemented with 0.05% Tween-20), and incubated with anti-GST-HRP antibody (GE Healthcare). After several washes, the membrane was subjected to chemiluminescent reaction using Western Lightning Plus-ECL detection kit (Perkin–Elmer).

2.6. In vitro GAP assays

HEK293 cells were transfected with either pRK5-myc, pRK5-mycCdGAP (1–515), pRK5-mycCdGAP(1-515)(SaSaa), pEGFPC1, pEGFPC1-CdGAP(1-820), or pEGFPC1-CdGAP(1-820)(SaSaa) using Lipofectamine 2000 as per manufacturer. Thirty-six hour post-transfection, myc-tagged or GFP-tagged proteins were immunoprecipitated using anti-myc or –CdGAP antibodies [20] as described previously [5]. The amount of immunoprecipitated myc-tagged CdGAP was estimated on Coomassie Blue-stained SDS–PAGE by comparison with different amount of purified bovine serum albumin. Immunoprecipitated GFP-tagged CdGAP was quantified using a Victor X3 2030 Multilabel Reader spectrofluorimeter (Perkin–Elmer). According to this estimation, 500 ng of immunoprecipitated CdGAP was used for the in vitro GAP assays, as described previously [5].

3. Results

To investigate the interaction between CdGAP and Intersectin-SH3D, we first determined whether the association is direct using an in vitro translation assay. A fragment corresponding to the N-terminal 515 amino acids of CdGAP was expressed as a ^{35}S-methionine-labeled protein and incubated in a GST pull-down assay using GST-Intersectin-SH3D or GST-actopaxin as a positive control. The focal adhesion protein actopaxin has previously been shown to interact with a region of CdGAP corresponding to the first 515 amino acids [19]. As shown in Fig. 1A, we found that CdGAP(1–515) binds to both Intersectin-SH3D and actopaxin but not GST, suggesting that the interaction between CdGAP and Intersectin-SH3D is direct. To further define the region of CdGAP binding to Intersectin-SH3D, protein lysates of HEK293 cells expressing various myc-tagged CdGAP deletion mutant proteins were incubated with GST-Intersectin-SH3D immobilized on glutathione-agarose beads (Fig. 1B and C). We found that Intersectin-SH3D was able to interact with CdGAP-(1–515), -(1–358), -(254–515), and -(181–358), all encompassing the BR region of CdGAP. On the contrary, CdGAP-(1–312) and CdGAP-(313–515) did not bind to Intersectin-SH3D. Therefore, these results show that an intact BR region is required to mediate the Intersectin-SH3D-CdGAP interaction.

To further define the amino acid motif within the BR region that mediates Intersectin-SH3D/CdGAP interaction, we used a peptide overlay assay comprising 20 overlapping peptides (15-mers), corresponding to the amino acid sequence of the BR region (249–358), immobilized on a cellulose membrane and incubated with purified GST-Intersectin-SH3D or GST proteins as a negative control (Fig. 2). Interestingly, we found that 8 consecutive peptides corresponding to residues 284–333 were able to specifically bind to Intersectin-SH3D with different affinities (Fig. 2). The interaction between peptide 10 (residues 294–308) and 14 (residues 314–328) with Intersectin-SH3D showed the strongest affinity. Interestingly, amino acid sequence analysis of the peptides revealed a common SKSK motif, suggesting that these residues are important for the interaction between Intersectin-SH3D and CdGAP.

Furthermore, a close analysis of the amino acid sequences of CdGAP and its protein homologs ARHGAP30, ARHGAP32/GRIT, and ARHGAP33/Noma-GAP revealed that the BR region is highly conserved amongst the CdGAP-related proteins (Fig. 3A). In particular,

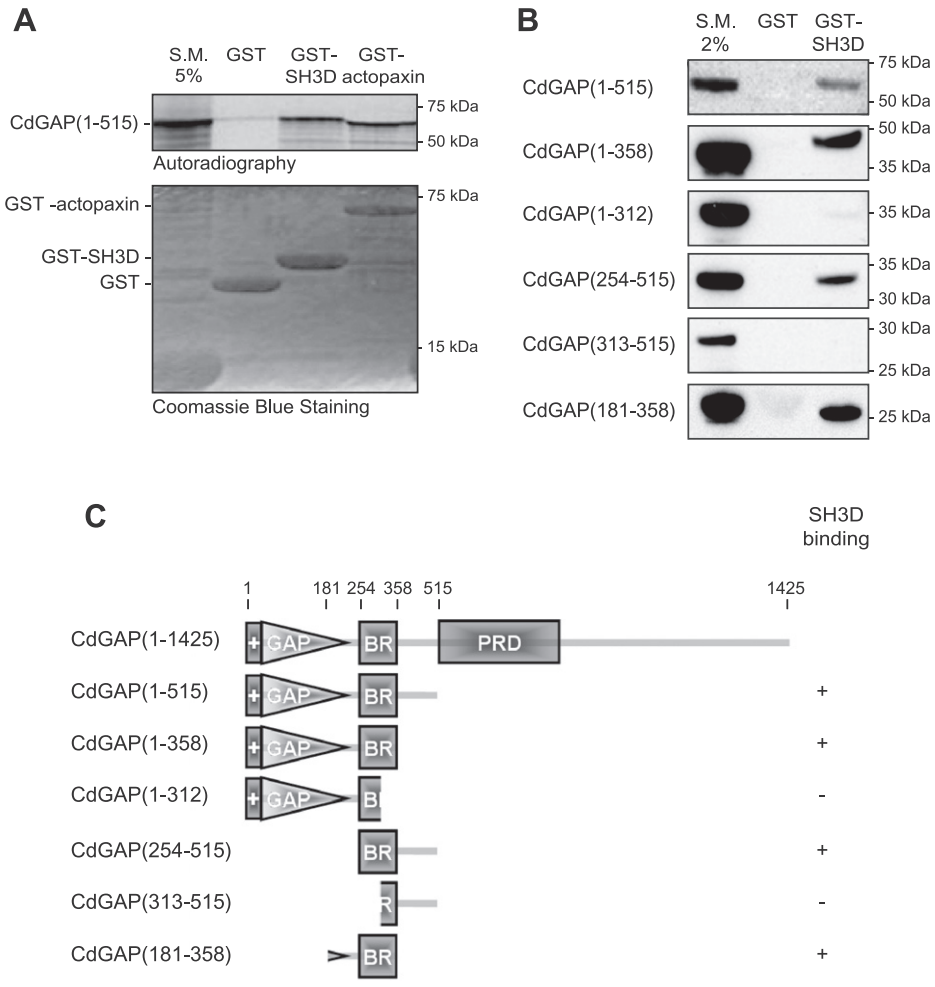
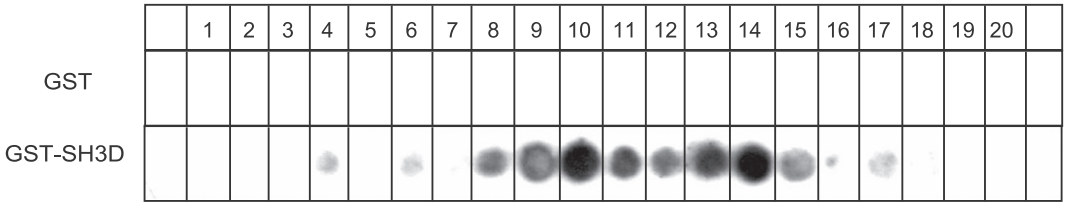


Fig. 1. An intact BR domain of CdGAP is required to bind to Intersectin-SH3D. (A) In vitro [^{35}S]-methionine translated CdGAP (1–515) was incubated with GST, GST-SH3D or GST-actopaxin coupled to glutathione-agarose beads in a GST pull down assay. 5% of the starting material (S.M.) was loaded in the first lane. (B) Myc-tagged deletion mutants of CdGAP expressed in HEK293 cells were incubated with GST or GST-SH3D proteins coupled to glutathione–agarose beads in a GST pull down assay. CdGAP was revealed by western blotting using anti-myc antibodies. 2% of starting material (S.M.) was loaded in the first lane. (C) Schematic representation of mouse full-length CdGAP (a.a. 1–1425) and CdGAP deletion mutants. +: polybasic residue cluster, GAP: GTPase-activating Protein, BR: basic-rich, PRD: proline-rich domain.

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247-LVSLEEAQARSLATNHPARKERRENSLPEIVPPPFHTVLELPDNKRKLSSKSKKWKSIFNLGRSGSDSKSKLSRNGSVFVRGQRLSVEKATIR-339
  8  ------------------------------------VLELPDNKRKLSSKS
  9  -----------------------------------------DNKRKLSSKSKKWKS
 10  ----------------------------------------------LSSKSKKWKSIFNLG
 11  ------------------------------------------------------KKWKSIFNLGRSGSD
 12  ------------------------------------------------------------IFNLGRSGSDSKSKL
 13  ------------------------------------------------------------------RSGSDSKSKLSRNGS
 14  ------------------------------------------------------------------------SKSKLSRNGSVFVRG
 15  ------------------------------------------------------------------------------SRNGSVFVRGQRLSV
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Fig. 2. Peptide overlay assay with GST-SH3D. The C-terminus of twenty 15-mer overlapping peptides corresponding to amino acids 249–358 of CdGAP were covalently bound to a cellulose membrane as per manufacturer. The membrane was incubated with GST or GST-SH3D proteins and bound proteins were revealed using an anti-GST-HRP antibody. Protein sequence alignment of peptides 8–15 is shown. Two distinct SKSK motifs are highlighted in bold.

the positively charged residues of the SKSK motif are present in all CdGAP-related proteins (Fig. 3A). To determine whether the BR domain of other CdGAP-related proteins is able to interact with Intersectin-SH3D, we examined the interaction between Intersectin-SH3D and ARHGAP30. Indeed, myc-tagged ARHGAP30 expressed in HEK293 cells was able to interact with Intersectin-SH3D

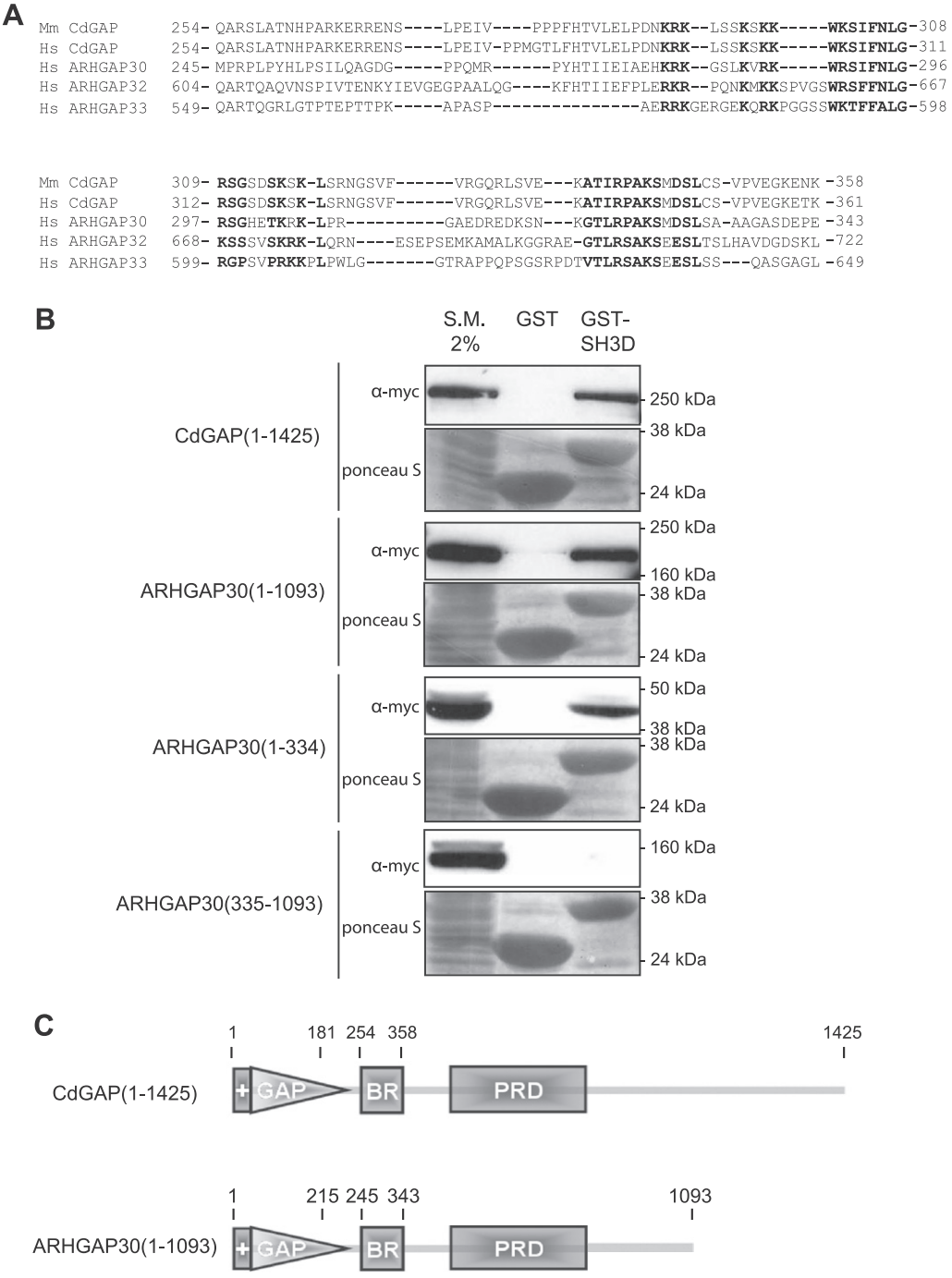

Fig. 3. Intersectin-SH3D interacts with the CdGAP-related protein ARHGAP30. (A) Amino acid alignment of the BR region of mouse and human CdGAP with protein homologs. Conserved amino acids are highlighted in bold. (B) Myc-tagged CdGAP, ARHGAP30, and ARHGAP30 deletion mutants were transiently expressed in HEK293 cells and protein lysates were incubated with GST or GST-SH3D proteins coupled with glutathione–agarose beads. GST pull down proteins were revealed by western blotting using anti-myc antibodies. 2% of starting material (S.M.) was loaded as a control. (C) Schematic representation of mouse full-length CdGAP (a.a. 1–1425) and ARHGAP30. +: polybasic residue cluster, GAP: GTPase-activating protein, BR: basic-rich, PRD: proline-rich domain.

(Fig. 3B). Moreover, ARHGAP30 (1–334) containing the N-terminus GAP domain followed by the BR region bound to Intersectin-SH3D whereas ARHGAP30 (335–1092) lacking the BR domain was not able to interact with Intersectin-SH3D (Fig. 3B and C). Thus, these results suggest that the conserved BR domain within CdGAP-related proteins mediates the interaction CdGAP/Intersectin-SH3D.

We then performed alanine-scanning mutagenesis to identify which residues in the BR region of CdGAP mediate the interaction with SH3D. Based on the amino acid sequence similarity between ARHGAP30 and CdGAP, CdGAP point mutants were generated by amino acid substitution of conserved residues (Fig. 4A). We found that the interaction between Intersectin-SH3D and CdGAP point mutant 2 was significantly impaired compared with the binding of intersectin-SH3D to other CdGAP point mutants (Fig. 4B and C). Interestingly, this CdGAP point mutant 2 has its positively charged lysines replaced by alanines in the first SKSKK motif, previously identified using the peptide overlay assay (Fig. 2). However, replacement of the lysines by alanines in the second SKSK motif (CdGAP point mutant 7) did not alter the interaction with Intersectin-SH3D. Altogether, these results show that the SKSKK

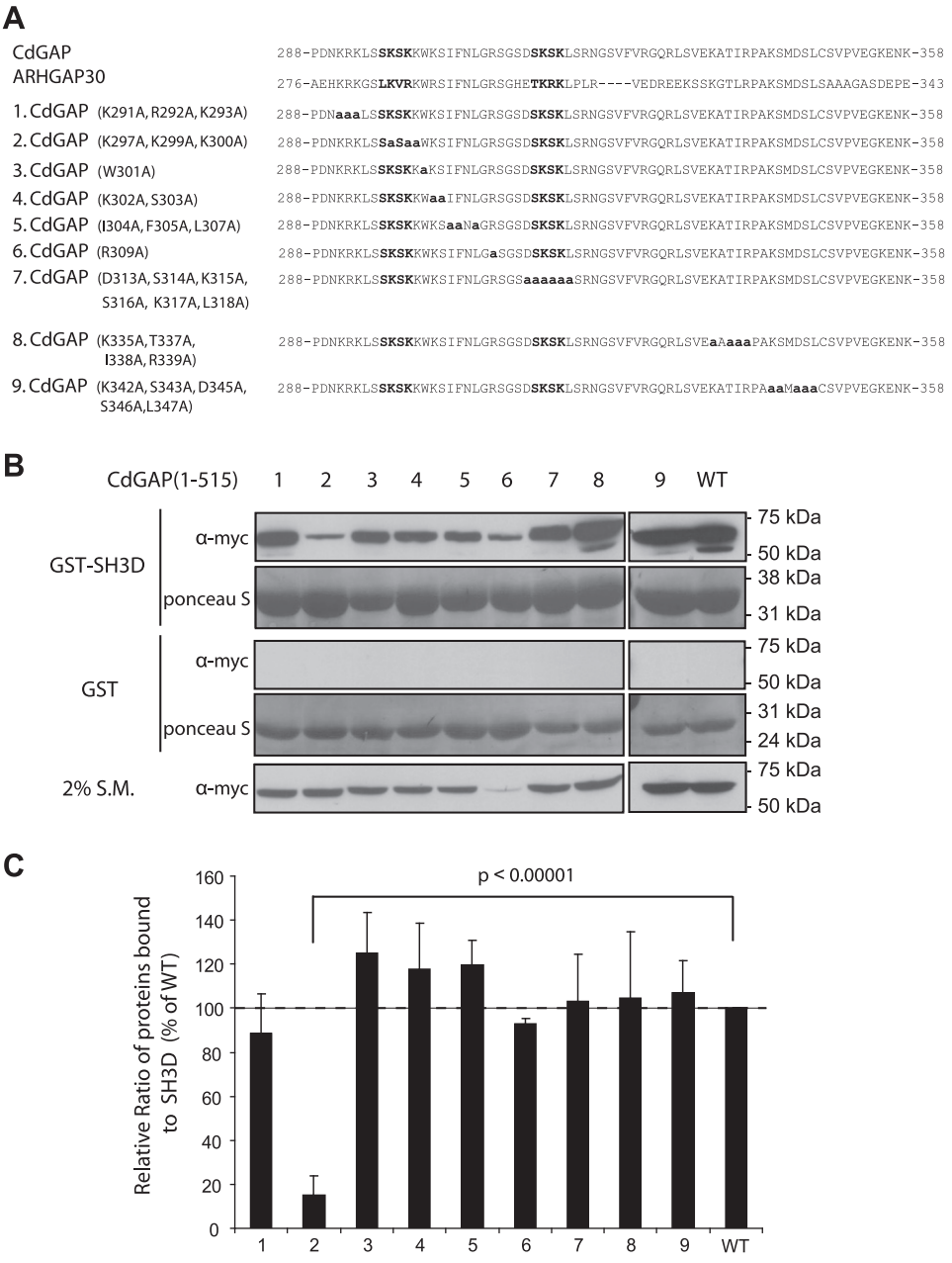

Fig. 4. The basic residues in the first SKSK motif are required for the interaction between CdGAP and Intersectin-SH3D. (A) Amino acid sequence alignment of the BR domains of CdGAP and ARHGAP30. CdGAP point mutants have been generated by alanine scanning mutagenesis based on the conserved residues between CdGAP and ARHGAP30. (B) Myc-tagged wild-type (WT) CdGAP (1–515) and CdGAP point mutants 1–9 expressed in HEK293 cells were incubated with GST or GST-SH3D proteins coupled to glutathione-agarose beads. Proteins specifically bound to the beads were revealed by western blotting using an anti-myc antibody. 2% of starting material (S.M.) was loaded as a control. (C) Quantitative analysis of blots as in (B), showing the relative ratio of CdGAP point mutants bound to GST-SH3D. A ratio of bound to total proteins was calculated for each myc-tagged protein and is represented relative to wild-type (WT) CdGAP (1–515). Error bars represent standard errors of the mean for at least five independent experiments. *P* value was determined by unpaired student's *t* test.

motif corresponding to residues 296–300 in the BR region of CdGAP is a novel SH3-binding sequence that mediates the interaction of CdGAP with the SH3D of Intersectin.

We then examined if the SKSKK motif is important for the regulation of CdGAP activity. In an in vitro GAP assay, [$Y^{32}P$]-GTP-loaded Rac1 was incubated with myc-tagged CdGAP(1–515) or CdGAP(1–515) (SaSaa) immunoprecipitated from HEK293 cell lysates. We observed a reduction in the GAP activity of CdGAP(1–515) (SaSaa) compared with the wild-type protein (Fig. 5A). However, when point mutations of the SKSKK motif were introduced into the full length CdGAP protein (1–820), the GAP activity of the mutant protein was inhibited (Fig. 5B). In the presence of GST-SH3D, the GAP activity of CdGAP(1–820) was reduced as previously shown [5] but it has no major effect on the mutant protein (Fig. 5C). Therefore, these results show that the SKSKK motif is important for the regulation of CdGAP activity in vitro.

4. Discussion

In this study, we have identified key amino acid residues involved in the direct binding of the SH3D domain of Intersectin with CdGAP. These findings suggest that evolutionary conserved lysine residues located within the BR region of CdGAP and its related protein homologs are central to a novel atypical SH3 binding motif xKx(K/R)K. It is well established that most of the SH3 domains characterized to date bind to conventional class I ([R/K]xXPxXP)

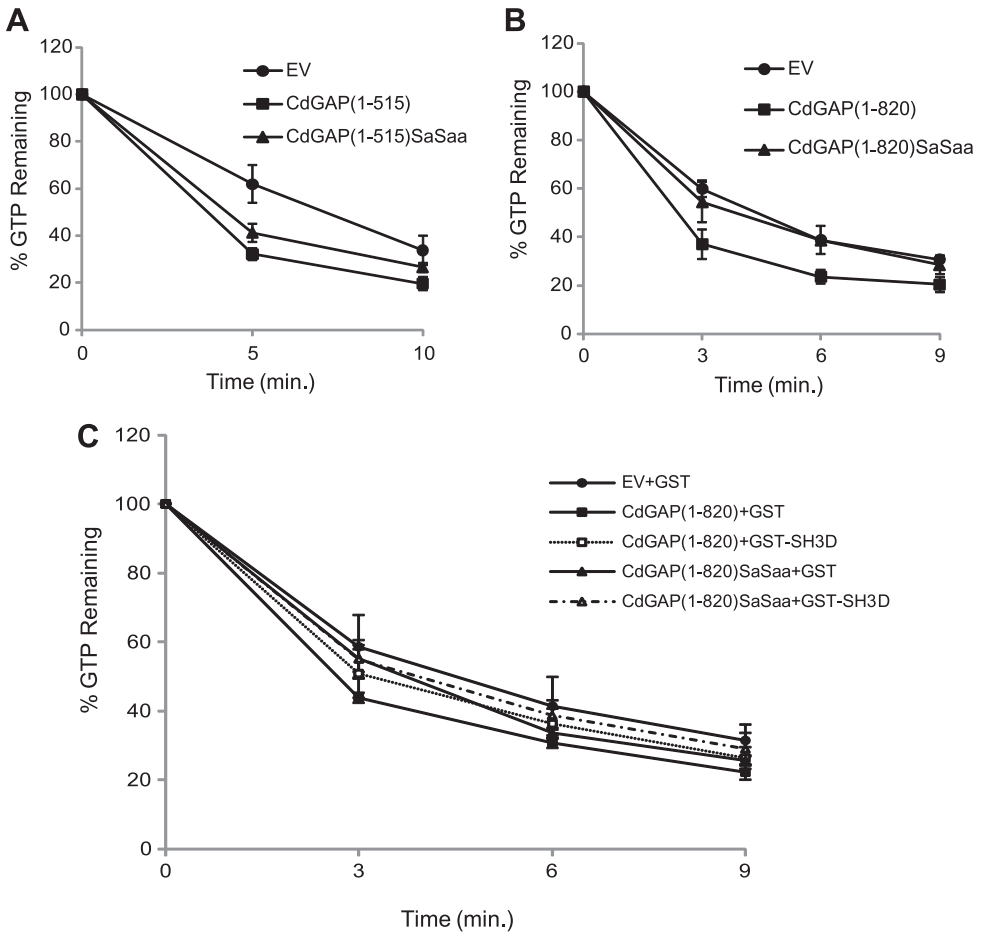

Fig. 5. The SKSKK motif is critical for the GAP activity of CdGAP in vitro. [γ-^{32}P]-GTP loaded Rac1 was incubated with myc-tagged immunoprecipitated CdGAP proteins (A) or with GFP-tagged immunoprecipitated CdGAP proteins (B and C) and a GAP assay was performed. (C) The in vitro GAP assay was performed in the presence of 10 μm GST or GST-SH3D. Error bars represent standard errors of the mean for at least three independent experiments.

or class II (XPxXPx[R/K]) peptide motifs [13,14]. However, a growing number of atypical motifs containing or not a proline residue have been discovered over the past years. These include the PxxxPR motif recognized by CIN85 in a number of proteins [15], the PxxDY motif that associates with Eps8 SH3 domain [16], the RKxxY motif in SKAP55 that mediates its interaction with the C-SH3 domain of SLAP [17], and the RxxK motif in SLP-76 bound by the C-SH3 domain of Gads [18]. Most of these motifs share with the novel identified xKx(K/R)K motif the presence of key basic residues. In contrast to other SH3 domains (A, B, C, and E) of Intersectin that interact with a large number of proteins though the consensus PXXP sequence [7–9], only CdGAP and the adaptor protein Numb have been shown to interact with the SH3D domain of Intersectin. It is noteworthy that the C-terminus of mouse Numb involved in the interaction with Intersectin-SH3D [11] also contains the residues SKSKQ, adding support to the findings that the novel xKx(K/R)K motif mediates the interaction with Intersectin-SH3D. Although the peptide overlay assay suggests that the second SKSK motif may be involved in the CdGAP/Intersectin-SH3D interaction (Fig. 2), the alanine scanning mutagenesis experiment shows that only the first SKSKK motif is essential to mediate the interaction with SH3D (Fig. 4). In this experiment, CdGAP proteins expressed in HEK293 cells may have post-translational modifications, absent in the synthesized peptides, that could alter the interaction with SH3D and account for the discrepancy between the two results. In support of these findings, we demonstrate that the critical SKSKK motif regulates the GAP activity of CdGAP in vitro. Indeed, the replacement of the lysine residues by alanines in the full-length protein CdGAP (1–820) is sufficient to inhibit the GAP activity. Interestingly, point mutations of the SKSKK motif in the shorter protein CdGAP(1–515) lacking the PRD slightly affect the GAP activity, suggesting that the PRD is required for this regulation. These data are in good agreement with our previous study showing that the PRD of CdGAP is required for Intersectin-mediated regulation of CdGAP activity [5].

In addition, it has been reported that the SH3 domains of Intersectin-1L regulate the GEF activity of Intersectin towards Cdc42 through intramolecular interactions with the adjacent DH-PH-C2 domains that do not involve proline peptide binding [11,22]. The interaction of Numb with Intersectin-SH3D relieves this autoinhibitory mechanism, resulting in a net activation of Cdc42 activity [11]. It will be of interest to determine whether CdGAP binding to the SH3D domain of Intersectin can also act as a positive regulator of the GEF activity of Intersectin-1L towards Cdc42. Combined with the negative regulation of CdGAP activity by Intersectin SH3 domains [5], these SH3 interactions would result in a net activation of Cdc42 activity. Future studies will determine how these molecular interactions are orchestrated in a spatial and temporal manner to affect Cdc42 activity in a physiological context.

Acknowledgments

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References

[1] Heasman, S.J. and Ridley, A.J. (2008) Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat. Rev. Mol. Cell Biol. 9, 690–701.

[2] Bos, J.L., Rehmann, H. and Wittinghofer, A. (2007) GEFs and GAPs: critical elements in the control of small G proteins. Cell 129, 865–877.

[3] Tcherkezian, J. and Lamarche-Vane, N. (2007) Current knowledge of the large RhoGAP family of proteins. Biol. Cell 99, 67–86.

[4] Lamarche-Vane, N. and Hall, A. (1998) CdGAP, a novel proline-rich GTPase-activating protein for Cdc42 and Rac. J. Biol. Chem. 273, 29172–29177.

[5] Jenna, S., Hussain, N.K., Danek, E.I., Triki, I., Wasiak, S., McPherson, P.S. and Lamarche-Vane, N. (2002) The activity of the GTPase-activating protein CdGAP is regulated by the endocytic protein intersectin. J. Biol. Chem. 277, 6366–6373.

[6] Pechstein, A., Shupliakov, O. and Haucke, V. (2010) Intersectin 1: a versatile actor in the synaptic vesicle cycle. Biochem. Soc. Trans. 38, 181–186.

[7] Hussain, N.K., Jenna, S., Glogauer, M., Quinn, C.C., Wasiak, S., Guipponi, M., Antonarakis, S.E., Kay, B.K., Stossel, T.P., Lamarche-Vane, N. and McPherson, P.S. (2001) Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP. Nat. Cell Biol. 3, 927–932.

[8] Yamabhai, M., Hoffman, N.G., Hardison, N.L., McPherson, P.S., Castagnoli, L., Cesareni, G. and Kay, B.K. (1998) Intersectin, a novel adaptor protein with two Eps15 homology and five Src homology 3 domains. J. Biol. Chem. 273, 31401–31407.

[9] Roos, J. and Kelly, R.B. (1998) Dap160, a neural-specific Eps15 homology and multiple SH3 domain-containing protein that interacts with Drosophila dynamin. J. Biol. Chem. 273, 19108–19119.

[10] Simpson, F., Hussain, N.K., Qualmann, B., Kelly, R.B., Kay, B.K., McPherson, P.S. and Schmid, S.L. (1999) SH3-domain-containing proteins function at distinct steps in clathrin-coated vesicle formation. Nat. Cell Biol. 1, 119–124.

[11] Nishimura, T., Yamaguchi, T., Tokunaga, A., Hara, A., Hamaguchi, T., Kato, K., Iwamatsu, A., Okano, H. and Kaibuchi, K. (2006) Role of numb in dendritic spine development with a Cdc42 GEF intersectin and EphB2. Mol. Biol. Cell 17, 1273–1285.

[12] Tong, X.K., Hussain, N.K., Adams, A.G., O'Bryan, J.P. and McPherson, P.S. (2000) Intersectin can regulate the Ras/MAP kinase pathway independent of its role in endocytosis. J. Biol. Chem. 275, 29894–29899.

[13] Mayer, B.J. (2001) SH3 domains: complexity in moderation. J. Cell Sci. 114, 1253–1263.

[14] Kaneko, T., Li, L. and Li, S.S. (2008) The SH3 domain – a family of versatile peptide- and protein-recognition module. Front Biosci. 13, 4938–4952.

[15] Kowanetz, K., Husnjak, K., Höller, D., Kowanetz, M., Soubeyran, P., Hirsch, D., Schmidt, M.H., Pavelic, K., De Camilli, P., Randazzo, P.A. and Dikic, I. (2004) CIN85 associates with multiple effectors controlling intracellular trafficking of epidermal growth factor receptors. Mol. Biol. Cell 15, 3155–3166.

[16] Mongiovi, A.M., Romano, P.R., Panni, S., Mendoza, M., Wong, W.T., Musacchio, A., Cesareni, G. and Di Fiore, P.P. (1999) A novel peptide-SH3 interaction. EMBO J. 18, 5300–5309.

[17] Kang, H., Freund, C., Duke-Cohan, J.S., Musacchio, A., Wagner, G. and Rudd, C.E. (2000) SH3 domain recognition of a proline-independent tyrosine-based RKxxYxxY motif in immune cell adaptor SKAP55. EMBO J. 19, 2889–2899.

[18] Liu, Q., Berry, D., Nash, P., Pawson, T., McGlade, C.J. and Li, S.S. (2003) Structural basis for specific binding of the Gads SH3 domain to an RxxK motif-containing SLP-76 peptide: a novel mode of peptide recognition. Mol. Cell 11, 471–481.

[19] LaLonde, D.P., Grubinger, M., Lamarche-Vane, N. and Turner, C.E. (2006) CdGAP associates with actopaxin to regulate integrin-dependent changes in cell morphology and motility. Curr. Biol. 16, 1375–1385.

[20] Tcherkezian, J., Danek, E.I., Jenna, S., Triki, I. and Lamarche-Vane, N. (2005) Extracellular signal-regulated kinase 1 interacts with and phosphorylates CdGAP at an important regulatory site. Mol. Cell Biol. 25, 6314–6329.

[21] Graham, F.L. and van der Eb, A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52, 456–467.

[22] Zamanian, J.L. and Kelly, R.B. (2003) Intersectin 1L guanine nucleotide exchange activity is regulated by adjacent src homology 3 domains that are also involved in endocytosis. Mol. Biol. Cell 14, 1624–1637.