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DOCK180, a Major CRK-Binding Protein, Alters Cell Morphology upon Translocation to the Cell Membrane

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CRK belongs to a family of adaptor proteins that consist mostly of SH2 and SH3 domains. Far Western blotting with CRK SH3 has demonstrated that it binds to 135- to 145-, 160-, and 180-kDa proteins. The 135- to 145-kDa protein is C3G, a CRK SH3-binding guanine nucleotide exchange protein. Here, we report on the molecular cloning of the 180-kDa protein, which is designated DOCK180 (180-kDa protein downstream of CRK). The isolated cDNA contains a 5,598-bp open reading frame encoding an 1,866-amino-acid protein. The deduced amino acid sequence did not reveal any significant homology to known proteins, except that an SH3 domain was identified at its amino terminus. To examine the function of DOCK180, a Ki-Ras farnesylation signal was fused to the carboxyl terminus of DOCK180, a strategy that has been employed successfully for activation of adaptor-binding proteins in vivo. Whereas wild-type DOCK180 accumulated diffusely in the cytoplasm and did not have any effect on cell morphology, farnesylated DOCK180 was localized on the cytoplasmic membrane and changed spindle 3T3 cells to flat, polygonal cells. These results suggest that DOCK180 is a new effector molecule which transduces signals from tyrosine kinases through the CRK adaptor protein.

v-Crk was identified originally as an oncogene product of the CT10 retrovirus and became the first example of an adaptor protein which consists mostly of SH2 and SH3 domains (24). The cellular homolog of v-Crk has been isolated from chickens, humans, and mice (22, 33, 36). Alternative splicing of the human *CRK* gene yields two forms of translation products, designated the 28-kDa CRK-I and 40- and 42-kDa CRK-II proteins (22). Microinjection of CRK induces neuronal differentiation of PC12 cells, and overexpression of v-Crk accelerates the neuronal differentiation of the PC12 cells induced by nerve growth factor and epidermal growth factor (EGF), which trigger the cognate tyrosine kinase receptors (13, 44). This CRK-dependent differentiation requires Ras, activation of which is enhanced by overexpression of CRK (20, 44). Moreover, two guanine nucleotide exchange proteins for the Ras family protein, mSos and C3G, have been shown to bind to the SH3 domain of CRK (20). These results have assigned CRK a position between receptor-type tyrosine kinases and the Ras family proteins in the signal transduction pathway.

CRK may also be involved in signalling from focal adhesions, which not only anchor cells to the extracellular matrix but also play a pivotal role in cell differentiation, migration, and proliferation (4, 16, 37). Binding of integrin to the extracellular matrix induces activation of focal adhesion tyrosine kinase bound to the cytoplasmic domain of the integrin β subunit. Activated and autophosphorylated focal adhesion tyrosine kinase, in turn, phosphorylates paxillin, a protein bound to focal adhesion tyrosine kinase and vinculin (40). Tyrosine phosphorylation of p130^{cas} is also induced by integrin engagement (31). Because paxillin and p130^{cas} are two of the three major phosphotyrosine-containing proteins in Crk-transformed cells and bind to the SH2 domain of Crk (21, 26), Crk seems to have an important role in signalling from focal adhesions.

The adaptor proteins, including Crk, Grb2/Ash, and Nck, perceive signals from a number of tyrosine kinases by the interaction of SH2 with phosphotyrosine-containing peptides (34). The signals are next transmitted to the proteins bound to the SH3 domains through proline-rich sequences (5, 34). By using far Western blotting, we and others have previously shown that the SH3 domain of CRK binds to 135- to 145-, 160-, and 180-kDa proteins (7, 44). The 135- to 145-kDa proteinsis designated C3G and has been shown to be a guanine nucleotide exchange protein for Rap1 (45). The identities of the 160- and 180-kDa proteins have not been reported. Other proteins known to bind to the SH3 domain of Crk include Sos (7, 20), Abl (8), and Eps15 (43).

A function of an adaptor protein is to recruit cytoplasmic enzymes bound to its SH3 domain to the cell membrane (34). Thus, the membrane targeting of the SH3-binding proteins mimics the activation of SH3-binding proteins. A typical example is Sos, a guanine nucleotide exchange protein for Ras, which binds to the SH3 domains of Grb2. Membrane targeting of Sos by addition of a myristylation or farnesylation signal increases the level of GTP-bound Ras without binding to Grb2 (1, 35). Recently, conditional membrane targeting and activation of Sos have been achieved by use of the chemical inducer of dimerization FK1012 (15).

Here, we report on the molecular cloning of the 180-kDa CRK-binding protein designated DOCK180. We found that expression of the membrane-targeted DOCK180 protein significantly altered cell morphology, suggesting that DOCK180 regulates cell structures upon the activation of tyrosine kinases.

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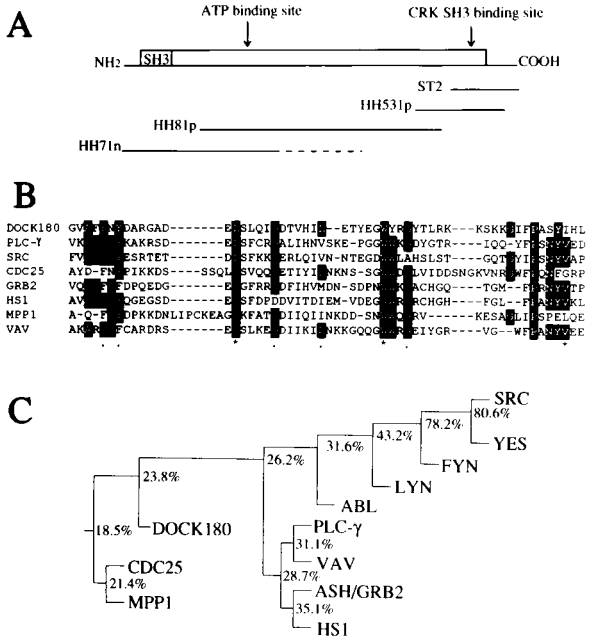

FIG. 1. Structure of DOCK180. (A) Schematic representation of DOCK180. The bars indicate cDNAs encoded by each recombinant λgt11-derived phage. The box represents the open reading frame. (B) Alignment of SH3 domains. Residues conserved in three or more proteins are in black boxes. (C) Dendrogram of SH3 domains. The dendrogram was constructed by CLUSTALV.

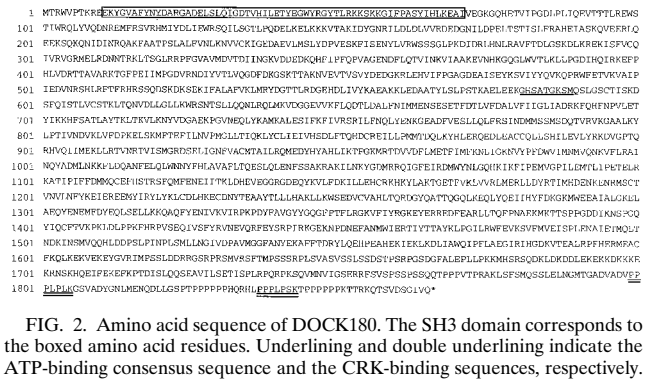

FIG. 2. Amino acid sequence of DOCK180. The SH3 domain corresponds to the boxed amino acid residues. Underlining and double underlining indicate the ATP-binding consensus sequence and the CRK-binding sequences, respectively.

MATERIALS AND METHODS

Screening of a cDNA library. An initially positive phage, ST2, was obtained by screening of a λgt11 cDNA expression library with glutathione *S*-transferase (GST)-CRK SH3 as the probe (44). To isolate the full-length cDNA of the ST2-encoded gene, we rescreened libraries derived from placenta, kidney, and neuroblastoma cells by using ^{32}P-labeled ST2 cDNA.

Sequence analysis. The cDNA was sequenced on both strands and across all cloning sites by use of pUC118 and pUC119 vectors. Dideoxy-chain termination sequencing reactions were carried out with reagents from ABI. Standard polyacrylamide sequencing gels were used, and products were analyzed with a DNA autosequencer (Perkin-Elmer-Cetus).

Construction of expression vectors. A cDNA fragment (positions 1 to 5727) containing the entire coding region was subcloned into the *Xho*I site of the pCAGGS eukaryotic expression vector to generate pCA-DOCK (30). The CAAX box region of human Ki-Ras2 cDNA was amplified by PCR with primer KRASCAX-F (5′-ATTCGAGGATCCTCTAGAAAGATGAGC-3′) which was designed to have an *Xba*I restriction site and corresponds to amino acid (aa) positions 163 to 171 of Ki-Ras2, and primer KRASCAX-R (5′-GAATTCGGATCCGTCGACTTACATAATTAC-3′), which has a *Bam*HI restriction site and corresponds to aa position 186 from the termination codon (27). The cDNA of the carboxyl-terminal region of DOCK180 was amplified by PCR with primers D1-14 (5′-GGGATCCTTCACGATGCCTTCCTCATCC-3′) and D-2 (5′-GCCTTGCGTCTAGACTGCACGATCCC-3′). In this amplified fragment, the termination codon was replaced with the *Xba*I restriction sequence, which we used to fuse the CAAX box sequence amplified as described above. An *Sph*I-*Bam*HI fragment of the amplified fragment covering the DOCK180 carboxyl terminus and the CAAX box was replaced with that of pCA-DOCK to generate pCA-DOCK-F.

Northern (RNA) blotting. Filters blotted with mRNAs from human adult and fetal tissues (Clontech Inc.) were incubated with ^{32}P-labeled probes at 42°C in the presence of 50% formamide, 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]), 1% sodium dodecyl sulfate (SDS), and 5× Denhardt's solution and then washed in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 50°C. Probes bound to the filter were analyzed by a FUJIX radioanalytic imaging system (Fuji Film Corp.).

Cells. COS1 (ATCC CRL-1651), 143B (ATCC CRL-8303), and A431 (ATCC CRL-1555) cells were maintained in Dulbecco's modified minimum essential medium supplemented with 10% fetal bovine serum. NIH 3T3 cells expressing the human EGF receptor were maintained as described previously (9). Molt-4 (ATCC CRL-1582), Raji (ATCC CCL-86), and THP-1 (ATCC TIB-202) cells were maintained in RPMI medium supplemented with 10% fetal bovine serum.

Antibodies. An *Eco*RI fragment of λgt11 clone ST2 (corresponding to aa 1810 to 1865; see Fig. 1A) was subcloned into pGEX1, a vector that yields a GST fusion protein (Pharmacia Biotechnology), to generate pGEX-ST2. The anti-DOCK180 sera used in this study were obtained from mice and rabbits immunized with the fusion protein expressed by pGEX-ST2. The antibodies against CRK, C3G, and GST used were described previously (22, 45).

Far Western blotting. Peptides containing the SH3 domains of CRK-I (aa 121 to 204) and GRB2/ASH (aa 1 to 217) were expressed in *Escherichia coli* as GST fusion proteins (22, 23). Far Western blotting with GST fusion proteins used as a probe was done as described previously (44).

Immunoprecipitation and Western blotting. Cells were lysed in lysis buffer (10 mM Tris-hydrochloride [pH 7.5], 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 500 μM Na_3VO_4, 10 mM NaF, 5 μg of aprotinin per ml, 1 mM phenylmethylsulfonyl fluoride). The DOCK180 and CRK proteins were immunoprecipitated with rabbit antisera and protein A-Sepharose. Proteins bound to the beads were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and analyzed by Western blotting with anti-CRK monoclonal antibody 3A8 and anti-DOCK180 mouse serum. The bound antibodies were detected by peroxidase-labeled anti-mouse immunoglobulin and with the enhanced-chemiluminescence system from Amersham.

Transfection and immunohistochemistry. Cells were grown on fibronectin-coated slide glasses before transfection. COS1 cells were transfected with pCAGGS-derived vectors by the DEAE-dextran method. NIH 3T3 cells were transfected with pCAGGS-derived vectors by use of Lipofectamine (GIBCO BRL). Cells were fixed with 100% ethanol 48 h after transfection and incubated with anti-DOCK180 diluted with 1% bovine serum albumin containing phosphate-buffered saline. Bound antibodies were detected with fluorescein isothio-

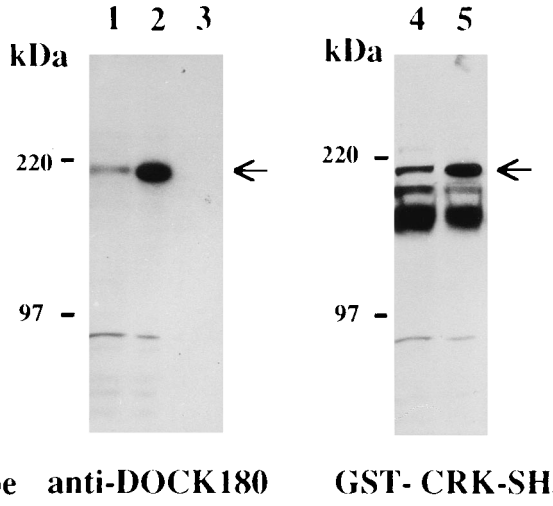

FIG. 3. The protein expressed from pCA-DOCK, a vector carrying the full-length DOCK180 cDNA, comigrates with the endogenous 180-kDa CRK-binding protein. COS1 cells transfected with pCAGGS (lanes 1 and 4) or pCA-DOCK180 (lanes 2, 3, and 5) were lysed in lysis buffer and immunoprecipitated with anti-DOCK180 rabbit serum (lanes 1, 2, 4, and 5) or normal rabbit serum (lane 3). Proteins were separated by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and probed with anti-DOCK180 or GST-CRK SH3 protein as described in the text. The arrows indicate DOCK180.

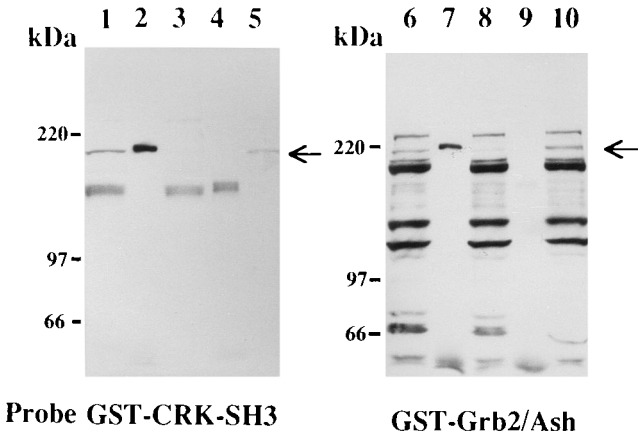

FIG. 4. Identification of DOCK180 among CRK- or Grb2/Ash-binding proteins. HeLa cell lysates (lanes 1 and 6) were divided into fractions that bound to anti-DOCK180 serum (lanes 2 and 7) and those that did not bind (lanes 3 and 8). Similarly, lysates were fractionated by anti-C3G serum into bound (lanes 4 and 9) and unbound (lanes 5 and 10) fractions. Proteins were separated by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and probed with either GST-CRK SH3 or GST-Grb2/Ash.

cyanate-conjugated anti-rabbit immunoglobulin antibody and analyzed by confocal microscopy (Carl Zeiss).

Nucleotide sequence accession number. The sequence data in this report have been submitted to the DDBJ/EMBL/GenBank libraries and assigned accession number D50857.

RESULTS

Isolation of DOCK180 cDNA. We have previously reported that the SH3 domain of CRK binds to cytoplasmic proteins of 135 to 145, 160, and 180 kDa (44). The cDNA of the 135- to 145-kDa protein, designated C3G, was isolated by screening of a cDNA expression library with GST-CRK SH3 as the probe (45). The same approach was employed successfully for isolation of the cDNA of the 180-kDa protein. The cDNA fragment of an initially positive clone, ST2, was used as a probe for rescreening of cDNA libraries derived from human placenta and NEC neuroblastoma cells. Among the positive cDNA clones, ST2, HH531p, HH81p, and HH71n were sequenced on both strands (Fig. 1A). The nucleotide sequences which did not overlap in these four cDNA clones were confirmed by the reverse transcriptase-mediated PCR method with the use of

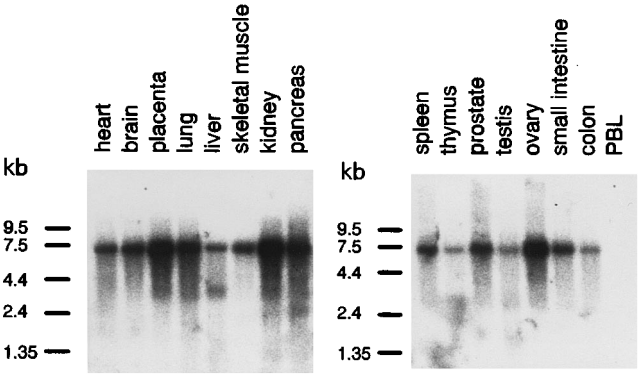

FIG. 5. Expression of DOCK180 mRNA in human adult tissues. Poly(A)$^+$ RNA extracts were separated by formaldehyde agarose gel electrophoresis and transferred to a nylon membrane. The filter was hybridized with DOCK180 cDNA labeled with [^{32}P]dCTP. PBL, peripheral blood leukocytes.

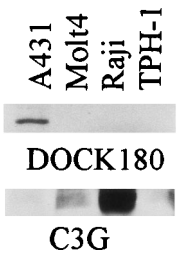

FIG. 6. Lack of expression of DOCK180 in lymphocyte and macrophage cell lines. The cell lines examined for DOCK180 expression were A431, an epidermoid carcinoma cell line; Molt4, a T-cell line; Raji, a B-cell line; and THP-1, a monocyte precursor cell line. Rapidly growing cells were lysed in lysis buffer (10 mM Tris-hydrochloride [pH 7.5], 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 500 μM Na_3VO_4, 10 mM NaF, 5 μg of aprotinin per ml, 1 mM phenylmethylsulfonyl fluoride). Ten micrograms of each soluble cell lysate was separated by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and blotted with anti-DOCK180 or anti-C3G rabbit serum, followed by a peroxidase-labeled antibody against rabbit immunoglobulin (Amersham). Bound antibodies were detected by the enhanced-chemiluminescence system.

total RNA of human 143B cells as the template. The combined cDNA sequence was 6,519 bp long and contained a 5,598-bp open reading frame that encodes a 1,866-aa protein with a calculated molecular mass of 215 kDa (Fig. 2). We designated this protein DOCK180, for 180-kDa protein downstream of CRK. The first ATG codon of DOCK180 meets Kozak's translation initiation criteria (19).

The CRK SH3-binding site was in the carboxyl terminus

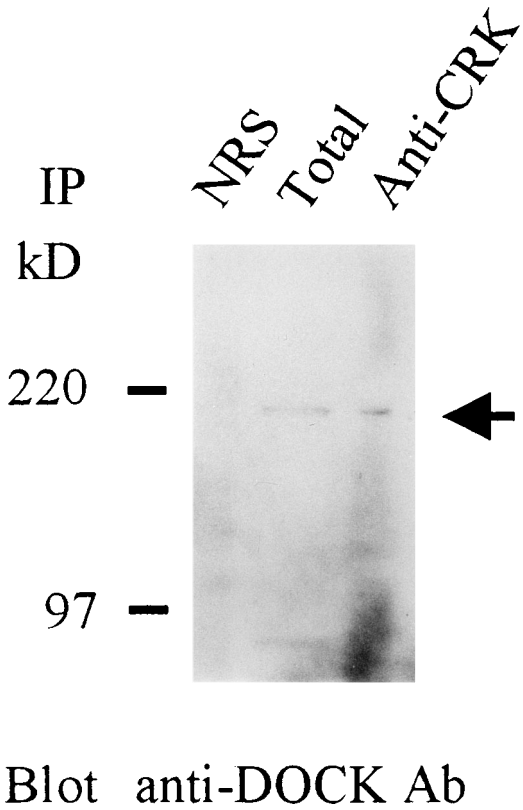

FIG. 7. Binding of CRK and DOCK180 in vivo. HeLa cell lysates were immunoprecipitated with normal rabbit serum (NRS) or anti-CRK rabbit serum (Anti-CRK). The total lane contained 1/20 of the amount of total cell lysates. Proteins were separated by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and probed with anti-DOCK180 mouse serum. IP, immunoprecipitation.

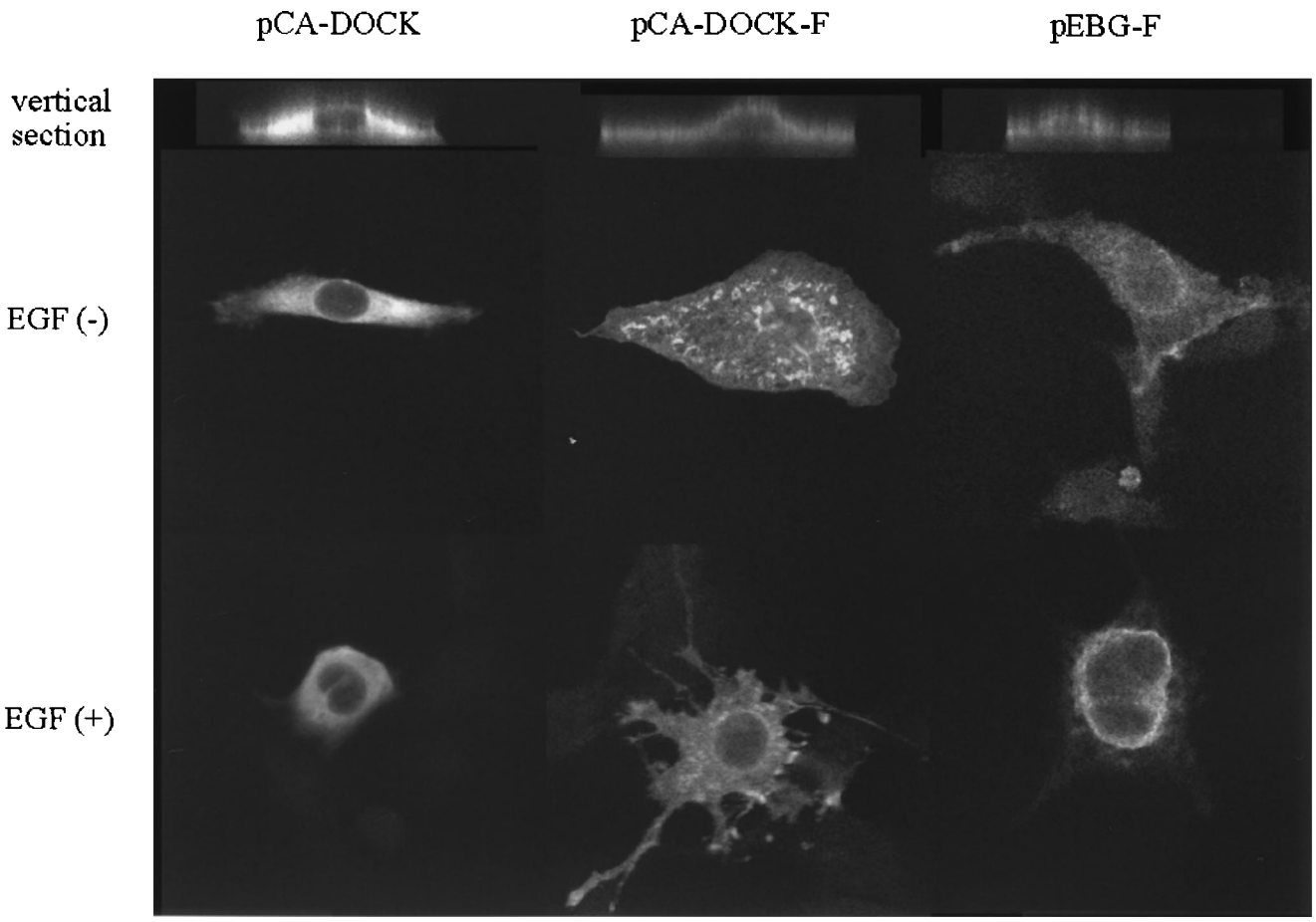

FIG. 8. Effect of overexpression of DOCK180, DOCK-F, or GST-F on the morphology of NIH 3T3 cells. NIH 3T3 cells expressing the human EGF receptor were transfected with pCA-DOCK for DOCK180, pCA-DOCK-F for DOCK-F, and pEBG-F for GST-F. Half of the cells were stimulated with 50 ng of EGF per ml for 16 h before fixation. Cells with or without EGF treatment were fixed 48 h after transfection; stained with anti-DOCK180 rabbit serum, followed by fluorescein isothiocyanate-labeled anti-rabbit goat immunoglobulin; and analyzed by confocal laser microscopy (Carl Zeiss). The upper panels show vertical sections of cells without EGF treatment.

(Fig. 2). We were able to identify two sequences that match the consensus sequence of the CRK SH3-binding domain, PPxLPxK (17), and confirmed that this region binds to the SH3 domain of CRK with high affinity (12).

A computer assisted sequence homology search of the GenBank and SwissProt databases did not reveal any significant homology, except for the amino-terminal region, which fulfilled the criteria of the SH3 domain (Fig. 1B) (29). The SH3 domain of DOCK180 was aligned with several other SH3 domains, and a dendrogram was constructed by use of CLUSTALV (14). The SH3 domain of DOCK180 is close to and shares 43% homology with that of HS1 (Fig. 1C). A domain search program, Prosite, identified an ATP-GTP-binding consensus sequence at aa 581 to 589 (Fig. 2).

Identification of DOCK180. We demonstrated that the DOCK180 cDNA encodes the CRK-binding 180-kDa protein as follows. Antisera to the carboxyl terminus of DOCK180 detected a 180-kDa protein on SDS-PAGE (Fig. 3, lane 1). Proteins expressed by pCA-DOCK, a plasmid containing the full-length DOCK180 cDNA, in COS1 cells comigrated with the 180-kDa protein detected by this anti-DOCK180 serum and with the 180-kDa protein detected with GST-CRK SH3, which was the probe (lanes 2 and 5). Products of in vitro translation also comigrated with the 180-kDa protein (12). When the DOCK180 protein was depleted from cell lysates by antisera, the 180-kDa protein was no longer detected by GST-CRK SH3 (Fig. 4, lanes 1 and 3). Similarly, the 135- to 145-kDa protein was removed by anti-C3G serum (Fig. 4, lanes 1 and 5). A replica of this filter was probed with GST-Grb2/Ash. Again, the 180-kDa band specifically disappeared after depletion by anti-DOCK180 serum (Fig. 4, lanes 6 and 8); however, the intensity of the 180-kDa band was significantly weaker than those of the 100-, 120-, and 170-kDa bands, which probably correspond to dynamin, Cbl, and hSos (6, 11, 28, 32). These results demonstrated that DOCK180 is the major 180-kDa protein bound to the SH3 domain of CRK.

Tissue distribution of DOCK180. The expression and distribution of DOCK180 mRNA were examined by Northern blotting (Fig. 5). A 7.4-kb transcript was expressed more strongly in the placenta, lungs, kidneys, pancreas, and ovaries, than in the thymus, testes, and colon. We could not detect DOCK180 mRNA in peripheral blood leukocytes, even after a long exposure. To further confirm that DOCK180 is not expressed in blood cells, expression of DOCK180 in Molt-4 T cells, Raji B cells, and THP-1 monocytes was examined (Fig. 6). DOCK180 was detected only in A431 epidermal cells, while C3G, another major CRK SH3-binding protein, was detected preferentially

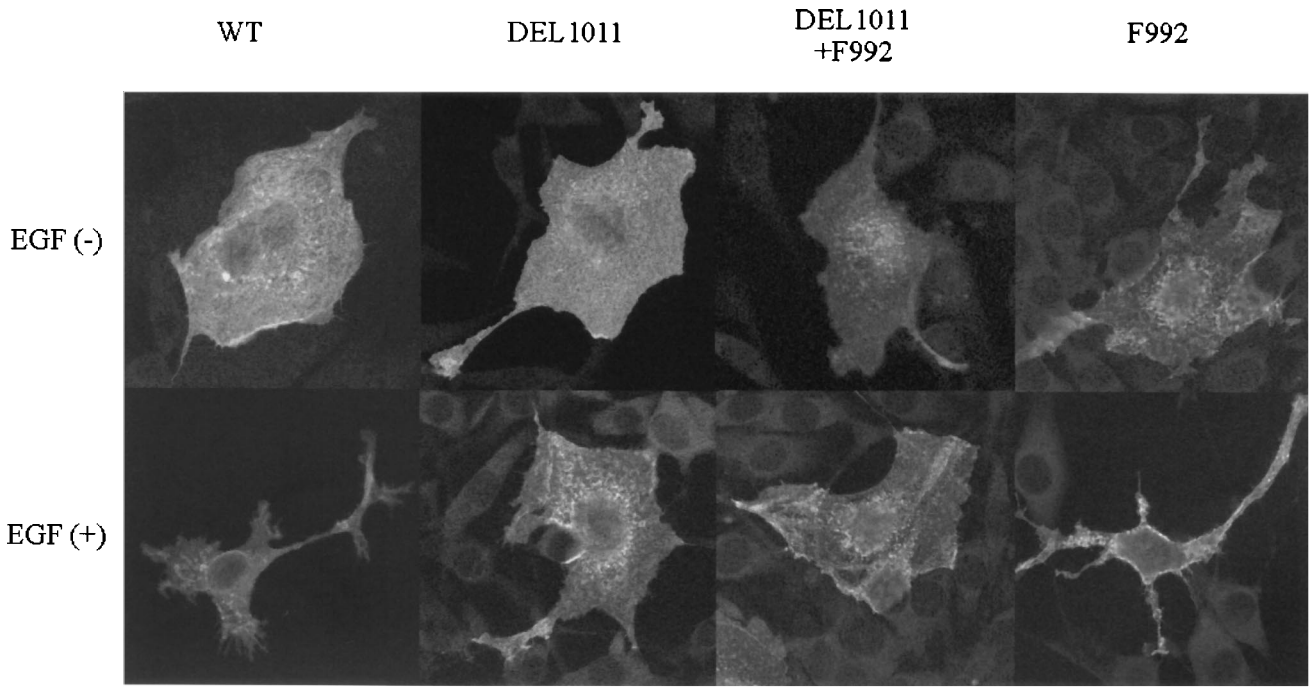

FIG. 9. Mutational analysis of EGF receptors. NIH 3T3 cells expressing wild-type (WT) and mutant (DEL1011, DEL1011+F992, and F992) EGF receptors were transfected with pCA-DOCK-F and stained with anti-DOCK180 rabbit serum as described in the legend to Fig. 8.

in Molt-4 and Raji cells. This suggests that DOCK180 is related to signalling of adherent cells.

DOCK180 binds to CRK in vivo. To examine whether DOCK180 binds to CRK in vivo, we immunoprecipitated proteins from HeLa cell lysates by using normal (Fig. 7, lane 1) or anti-CRK (lane 3) rabbit serum. DOCK180 was specifically associated with the anti-CRK immune complex. Because the total cell lysate lane (lane 2) contained only 1/20 of the amount in each of those containing the immunoprecipitates, the stoichiometry of binding was at least 5%.

DOCK180 fused to CAAX box of Ki-Ras induces morphologic changes in NIH 3T3 cells. A function of adaptor molecules is to recruit the cytoplasmic proteins bound to their SH3 domains close to the membrane. On the basis of this hypothesis, we constructed an expression plasmid for expression of a recombinant DOCK180 protein fused to the carboxyl terminus of Ki-Ras that contains the CAAX farnesylation signal. Wild-type DOCK180 localized diffusely in the cytoplasm and did not induce any remarkable change in NIH 3T3 cells. Farnesylated DOCK180 (Fig. 8), which will be referred to as DOCK-F, was localized mostly on the membrane. Moreover, expression of DOCK-F changed spindle 3T3 cells into flat, polygonal cells. Recombinant farnesylated GST (GST-F) was also localized on the membrane; however, we did not observe a morphologic change in these GST-F-expressing cells, indicating that the CAAX box alone does not alter cell morphology.

The NIH 3T3 cells used in this experiment express the human EGF receptor. Upon incubation with EGF, cells expressing wild-type DOCK180 or GST-F showed a typical morphologic transformation: rounding of cells and increased refractivity (Fig. 8). In contrast, cells expressing DOCK-F exhibited a dendritic cytoplasm after EGF treatment.

Effect of the autophosphorylation sites of the EGF receptor on the DOCK-F-induced morphologic change. For a better understanding of the effect of DOCK-F on EGF-induced morphologic change, we utilized a series of EGF receptor mutants which lack autophosphorylation sites but still transduce mitogenic signals (Fig. 9). The DEL1011 mutant lacks the carboxyl-terminal region to residue 1011, which contains three major autophosphorylation sites. In the F992 mutant, another autophosphorylation site, Tyr-992, was replaced with Phe. DEL1011+F992 has both mutations. Upon EGF treatment, 3T3 cells expressing DEL1011 or DEL1011+F992 exhibited a mild morphologic change compared with those expressing the wild-type and F992 mutant EGF receptors. We transfected the DOCK-F expression vector into these cell lines and observed the effect of EGF. The dendritic morphologic change after EGF treatment was induced in cells expressing the wild-type or F992 EGF receptor but not in those expressing the DEL1011 or DEL1011+F992 mutant EGF receptor. In cells expressing DEL1011 or DEL1011+F992, EGF transduces the mitogenic signal but does not evoke a fully transformed phenotype (9). Thus, the EGF-induced dendritic morphologic change in DOCK-F-expressing cells is understood as a conflict between the two signals; a signal from the activated EGF receptor reorganizes the cytoskeleton and renders cells round, while a signal from DOCK-F keeps cells flat.

DISCUSSION

This report describes a newly identified CRK-binding protein, DOCK180, that alters cell morphology upon translocation to the membrane. Amino acid sequence analysis of DOCK180 has identified an SH3 domain at the amino terminus and two CRK-binding sequences at the carboxyl end. The SH3 domain is often identified in proteins that are involved in signal transduction or associated with the cytoskeleton (5, 34). Whereas the former group of proteins also contains the SH2 domain, the latter possesses only SH3. The sequence alignment also suggested that the SH3 domain of DOCK180 resembles those

of cytoskeleton-associated proteins more closely than those of SH2-containing proteins.

Addition of a farnesylation or myristylation signal to the SH3-binding proteins has been employed successfully to mimic the activation of these proteins, including Sos and C3G (1, 10, 35). The morphologic change induced by DOCK-F was not observed in cells expressing wild-type DOCK180 or GST-F, indicating that the phenomenon was due to the recruitment of DOCK180 to the membranes. Because another major CRK SH3-binding protein, C3G, can also be activated by the farnesylation signal, the morphologic change induced by DOCK-F appears to reflect the physiological function of DOCK180. The substrate of Sos, Ras, localizes exclusively beneath the membrane, thus providing the reason for regulation of the enzymes by translocation. Therefore, the fact that DOCK-F, but not wild-type DOCK180, altered cell morphology strongly suggests that the DOCK180-interacting molecule(s) is in or on the membrane. The identification of the ATP-binding site implies that DOCK180 belongs to a new class of enzymes.

The mechanism by which the *crk* oncogene transforms cells remains unclear, even after the identification of the proteins bound to its SH3 domain. DOCK-F inhibited the morphologic transformation of NIH 3T3 cells induced by EGF, suggesting that DOCK180 antagonizes transformation. Recently, another CRK SH3-binding protein, C3G, has been shown to be a guanine nucleotide exchange factor for Krev-1/Smg21/Rap1 that was originally reported to be an anti-Ras gene (10). Moreover, expression of farnesylated C3G reverses the Ras-induced transformation of NIH 3T3 cells, as does Krev-1/Smg21/Rap1. C3G and DOCK180 are the two major effectors of CRK (7, 44); therefore, CRK itself might have antioncogenic activity. An increased quantity of the v-Crk protein is regarded as the major cause of transformation by v-Crk (36). The point mutations in the SH3 domain of v-Crk decrease its affinity for C3G and enhance its transforming activity (18, 36). This result suggests that v-Crk is a negative mutant dominant over c-Crk. However, a previous report that both the SH2 and SH3 domains are required for the transforming activity of v-Crk does not support this hypothesis (25). Activation of the minor component of the SH3-binding proteins, including Sos, might dominate over the antigrowth effect of C3G and DOCK180 in v-Crk-transformed cells (20).

Grb2/Ash and Crk, which were originally identified as adaptor molecules between the receptor-type tyrosine kinases and cytoplasmic enzymes, are now also known to transduce signals at focal adhesions (3, 41, 42). The SH2 domain of Crk binds to several phosphotyrosine-containing proteins, such as paxillin, p130^{cas}, pp120 in T cells, Shc, and the EGF receptor (2, 20, 21, 38, 39). Two of them, paxillin and p130^{cas}, are phosphorylated on tyrosine residues upon integrin stimulation (31, 41). Thus, at least part of the cellular Crk protein should participate in signalling from focal adhesions. Identification of the DOCK180 protein that binds to Crk and regulates cell morphology may reveal a new role of Crk in focal adhesions.

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