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# p130$^{Cas}$, an Assembling Molecule of Actin Filaments, Promotes Cell Movement, Cell Migration, and Cell Spreading in Fibroblasts

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**p130$^{Cas}$ (Cas) is an adaptor molecule which becomes tyrosine phosphorylated by v-Src- or v-Crk-triggered transformation and several physiological stimuli, such as cell attachment to fibronectin. We previously generated mice lacking Cas and demonstrated that Cas functions as an assembling molecule of actin filaments. To further explore Cas role in cellular function, we established Cas-deficient and Cas-re-expressing fibroblasts and compared their behaviors in response to several biological stimuli. We found that Cas-deficient fibroblasts showed significant defects in cell movement after mechanical wounding and in cell migration toward fibronectin as compared with Cas-re-expressing cells. In addition, when plated on fibronectin-coated dishes, Cas-deficient cells exhibited a significant delay in cell spreading as compared with Cas-re-expressing cells albeit that protein-tyrosine phosphorylation was similarly induced. These results demonstrated that Cas functions as a molecule promoting cell movement, cell migration, and cell spreading and suggest that Cas would be implicated in various physiological and pathological processes, such as would healing, chemotaxis, and tumor invasion.** 

Cells attach to the extracellular matrix (ECM) through cell surface receptors, integrins [1]. The attachment sites of the cells are composed of multiprotein complexes, called focal adhesions [2], where actin stress fibers terminate and many cytoskeletal and signaling molecules cluster. Cell-ECM interaction transmits extracellular signals into cell interior through integrins [1, 3] and the intracellular signaling has been shown to involve tyrosine phosphorylation of intracellular proteins, such as Fak [1, 4], paxillin [5], tensin [6], cortactin [7], mitogen-activated protein (MAP) kinases [8], and p130$^{Cas}$ [7, 9].

p130$^{Cas}$ (Cas, Crk-associated substrate) was originally identified as a highly tyrosine phosphorylated 130 Kd protein in cells transformed by v-Crk and v-Src oncoproteins [10–12]. We purified rat Cas by two-step immunopurification procedures and cloned *cas* cDNA using partial peptide sequences [13, 14]. The predicted amino acid sequences of Cas revealed that it is an adaptor molecule with a unique structure that is composed of an SH3 region at the N-terminus, 15 repeats of YXXP motifs in the trunk, and several tyrosine residues and a proline-rich region near the C terminus [13]. Studies have shown that Cas becomes tyrosine phosphorylated following various physiological stimuli, such as cell adhesion [7, 9], cytokine receptor engagement [15, 16], and growth factor stimulation [17, 18]. In addition, Cas has been proven to interact with a number of cellular proteins, such as Fak [19], Src [20], Crk [21], CrkL [22], Nck [23], PTP1B [24], and PTP-PEST [25]. These results indicate that Cas contributes to cellular signaling through forming complexes with specific target proteins. To clarify the *in vivo* roles of Cas, we generated mice lacking Cas by homologous recombination [26]. We found that Cas functions as an assembling molecule of actin filaments and plays essential roles in myofibril and Z-disk formation in the heart, actin stress fiber formation in primary fibroblasts, and Src-induced morphological transformation and anchorage-independent cell growth in soft agar [26]. Although these results demonstrate that Cas is required for embryonic development, cytoskeletal organization, and oncogene-induced transformation, the roles of Cas in cellular function have not fully been understood. To address this issue, we established Cas-deficient and Cas-re-expressing primary fibroblasts and examined their behaviors in response to several biological stimuli.

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## MATERIALS AND METHODS

*DNA transfection and cell cloning.* Rat *cas* cDNA [13] was subcloned into an expression vector, bsr/pSSR$\alpha$, which contains a blasticidin-resistance gene. The resultant plasmid was transfected into Cas-deficient cells and the transfected cells were subjected to blasticidin selection as described earlier [26]. Colonies resistant to blasticidin were picked up, expanded, and subjected to Western blot analysis for the re-expression of Cas.

*Western blot analysis.* Proteins were extracted by lysing cells in the RIPA lysis buffer as described [13]. Protein aliquots were separated by SDS-PAGE and probed with 1:2000 diluted anti-Cas antibody [13] or anti-phosphotyrosine antibody, 4G10 [27]. Positive signals were visualized using the ProtoBlot Western AP system (Promega).

*Wound healing assay.* Wound healing motility assay was performed essentially as described previously [28]. In brief, confluent cells were scraped with a 27-gauge needle to create a 'wound', detached cells were removed by washing with PBS, and the remaining cells were allowed to migrate to close the wound in a fresh medium.

*Cell migration assay.* Cell migration assay was performed using modified Boyden chambers [29], in which the top wells were separated from bottom wells by a polycarbonate membrane (tissue culture-treated, 6.5 mm-diameter, 10 $\mu$m-thickness, and 8 $\mu$m-pore, Transwell). Growing cells were trypsinized, washed, suspended in 0.1% BSA in DMEM, and added to the upper side of the membrane in the top wells. Cells were then allowed to migrate through the membrane and to reach the lower side of the membrane in the absence or presence of fibronectin (FN, 10 $\mu$g/ml), which had been added to the bottom well. After 6 hours' incubation, non-migratory cells on the upper side of the membrane were wiped and migrated cells on the lower side of the membrane were fixed, stained, and counted.

*Cell spreading assay.* Cell spreading assay was performed as described previously [30]. In brief, cells were overnight serum-starved, trypsinized, and re-plated on FN-coated dishes (10 $\mu$g/ml). The spreading ratio of the cells were counted as described [30].

## RESULTS

*Establishment of Cas-re-expressing fibroblasts.* To establish fibroblasts that re-express Cas, Cas-deficient fibroblasts were transfected with bsr/Cas and transfected cells were subjected to selection with bsr. Re-expression of Cas in blasticidin-resistant clones was examined by Western blot with anti-Cas antibody [13] (Fig. 1A). Three independent clones that re-express Cas at a comparable level to wild-type cells were chosen and used for further analyses.

*Re-expression of Cas restored morphological and cytoskeletal abnormalities.* We investigated whether Cas-re-expressing cells showed restoration of abnormalities observed in Cas-deficient fibroblasts [26]. Fig. 1B shows representative morphological appearances of Cas-deficient and Cas-re-expressing cells. Cas-deficient fibroblasts were flat and round in shape (left), while Cas-re-expressing cells exhibited an elongated and spindle-shaped appearance (right), which closely resembles to that of wild-type cells [26]. Thus, re-expression of Cas rescued the morphological abnormality observed in Cas-deficient cells. In addition, immunofluorescent staining with phalloidin showed that Cas-re-expressing cells restored impaired actin bundling in Cas-deficient cells and exhibited long actin stress fiber formation as observed in wild-type cells (data not shown). These results demonstrated that re-expression of Cas corrected the morphological and cytoskeletal abnormalities observed in Cas-deficient cells and exhibited normal phenotypes similar to wild-type cells.

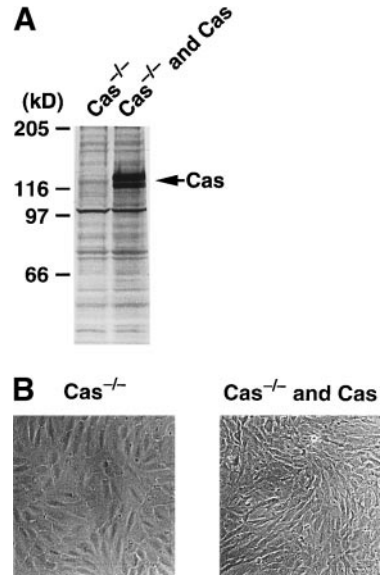


**FIG. 1.** (A) Western blot for detecting the re-expression of Cas. Sixty $\mu$g of cell lysates extracted from Cas-deficient ($Cas^{-/-}$) and Cas-re-expressing ($Cas^{-/-}$ and Cas) fibroblasts were separated by 7.5% SDS-PAGE, blotted to nitrocellulose membrane, and probed with anti-Cas antibody [13]. The position of Cas is indicated by an arrow and the positions of protein markers are shown on the left. (B) Morphological appearances of Cas-deficient ($Cas^{-/-}$) and Cas-re-expressing ($Cas^{-/-}$ and Cas) fibroblasts. While Cas-deficient cells showed a morphologically abnormal appearance (flat and round-shaped), Cas-re-expressing cells restored a normal phenotype (elongated and spindle-shaped).

*Cas promoted cell movement.* To investigate functional defect(s) caused by Cas-deficiency, Cas-deficient and Cas-re-expressing cells were subjected to the wound healing motility assay [28], which detects non-chemotaxic and horizontally migrating ability of the cells. The healing processes at various periods of time (0, 3, 6, and 12 hrs) after wounding are shown in Fig. 2A and the mean percentages of the filled area at each time period are shown in Fig. 2B. As shown in these figures, Cas-re-expressing cells moved much faster and covered the wounded area more efficiently as compared with Cas-deficient cells. At 12 hours after wounding, Cas-re-expressing cells had moved to fill ~70% of the gap and the cells migrating from both ends of the wound had begun cell-cell contact, while only ~30% of the area was filled by Cas-deficient cells. These findings indicated that re-expression of Cas significantly promoted cell movement.

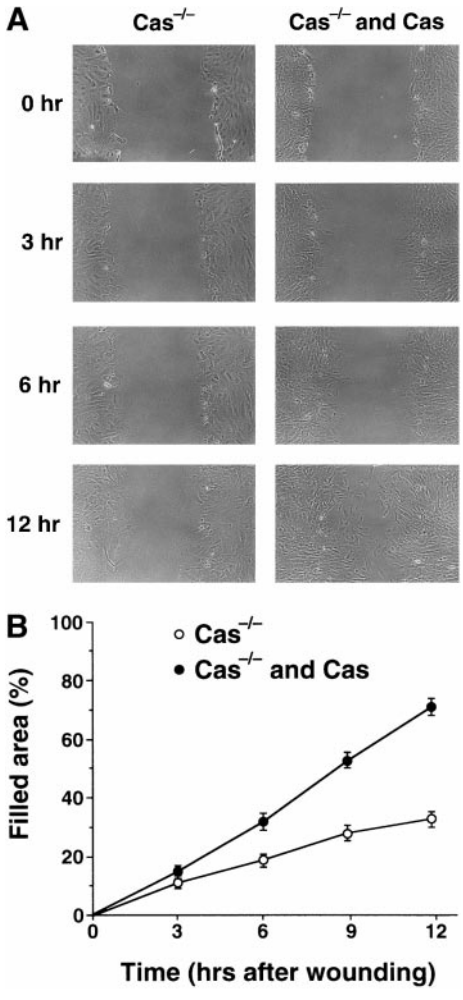


**FIG. 2.** Wound healing motility assay. (A) Photographs of Cas-deficient (Cas$^{-/-}$) and Cas-re-expressing (Cas$^{-/-}$ and Cas) cells at 0, 3, 6, and 12 hours after wounding. The Cas-re-expressing cells show faster moving rate than Cas-deficient cells and heal the wound more efficiently. (B) Percentages of the filled area at each time period. The mean percentage of three experiments using independent clones are shown. White and black circles indicate Cas-deficient (Cas$^{-/-}$) and Cas-re-expressing (Cas$^{-/-}$ and Cas) cells, respectively, and error bars show standard deviation.

*Cas promoted cell migration toward fibronectin.* We next examined whether the re-expression of Cas affected chemotaxic and invasive ability of the cells. Cas-deficient and Cas-re-expressing cells were subjected to the cell migration assay using modified Boyden chambers [29] with or without FN. The representative photographs of cells that have migrated through the membrane are shown in Fig. 3A and the mean counts of the migrated cells are shown in Fig. 3B. As shown in these figures, in the absence of FN (−FN), the number of Cas-re-expressing cells that had migrated through the membrane was ~1.5-fold higher that of Cas-deficient cells (Fig. 3A upper panels and Fig. 3B left), which is consistent with the results obtained by the would healing motility assay (Fig. 2A and 2B). In contrast, when FN was added (+FN), Cas-re-expressing cells gained a significant increase in the migratory ability as compared with Cas-deficient cells (Fig. 3A lower panels and Fig. 3B, right). Although FN promoted the migrating rate in both types of cells, the number of migrated Cas-re-expressing cells increased up to ~4-fold, whereas Cas-deficient cells showed only ~1.4-fold increase (Fig. 3B). These findings demonstrated that the presence of Cas significantly promoted the FN-mediated cell migratory ability.

*Cas promoted cell spreading without affecting protein-tyrosine phosphorylation.* We finally examined the roles of Cas in cell attachment and cell adhesion to FN. Both types of cells attached to the dishes soon after plating and no difference was observed in their attachment capacities (data not shown). However, after attachment, Cas-deficient cells exhibited a significant delay in cell spreading as compared with

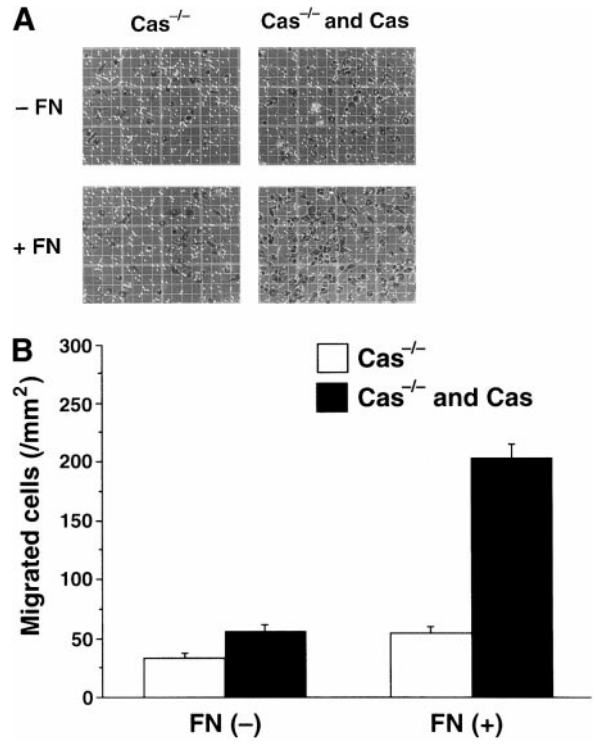


**FIG. 3.** Cell migration assay. (A) Photographs of Cas-deficient (Cas$^{-/-}$) and Cas-re-expressing (Cas$^{-/-}$ and Cas) cells that have migrated through the membrane of the chamber. −FN and +FN indicate the absence and presence of FN, respectively. (B) The numbers of the cells that have migrated through the membrane. The mean cell counts (per 1 mm$^2$) in several selective fields in three experiments using independent clones are shown. In the absence of FN (−FN), Cas-re-expressing cells showed ~1.5-fold faster migratory rate as compared with Cas-deficient cells (left). In contrast, in the presence of (+FN), Cas-re-expressing cells gained significant increase in the migratory ability and migrated ~4-fold faster than Cas-deficient cells (right). White and black bars indicate Cas-deficient (Cas$^{-/-}$) and Cas-re-expressing (Cas$^{-/-}$ and Cas) cells, respectively, and error bars show standard deviation.

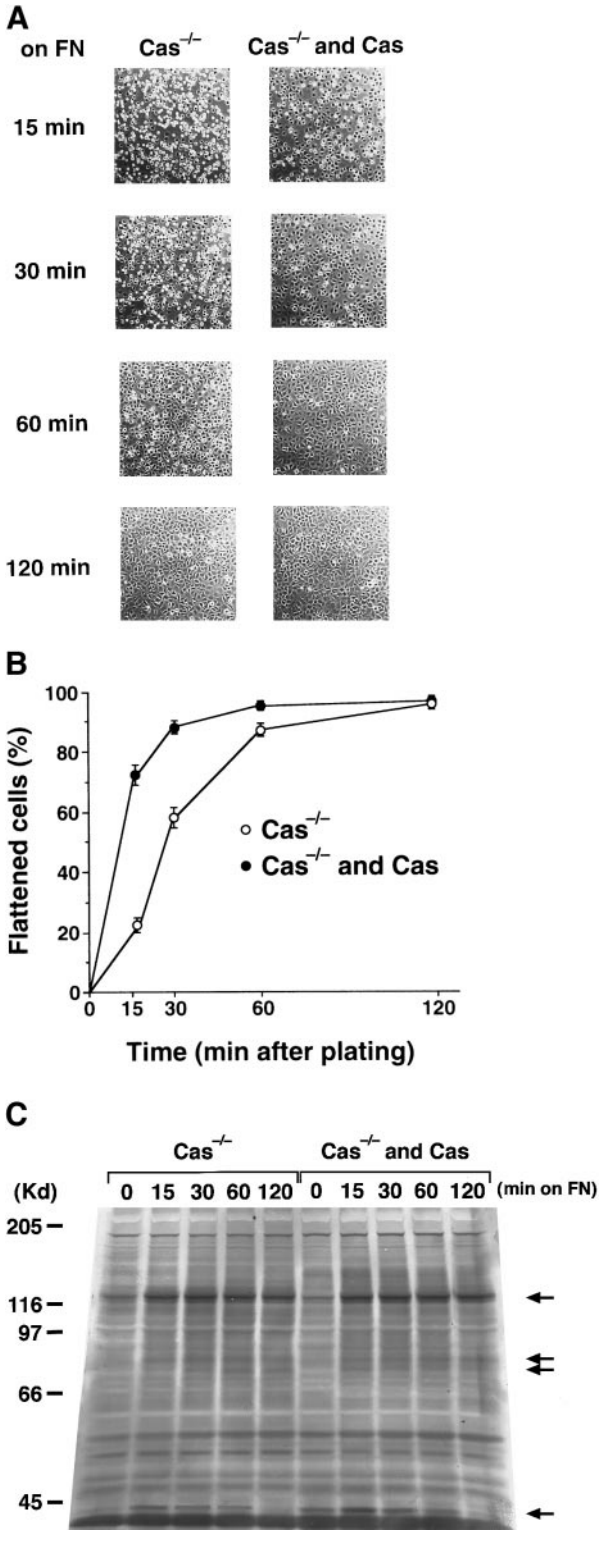


**FIG. 4.** Cell attachment and adhesion to FN. (A) Photographs of Cas-deficient ($Cas^{-/-}$) and Cas-re-expressing ($Cas^{-/-}$ and Cas) cells at 15, 30, 60, and 120 min after plating on FN-coated dishes (10 $\mu$g/ml). Cas-deficient cells show a significant delay in cell spreading as compared with Ca-re-expressing cells. (B) Cell spreading was quantitated by calculating the percentages of flattened cells. The mean percentages in several selective fields in two experiments using independent clones are shown. White and black circles indicate Cas-deficient ($Cas^{-/-}$) and Cas-re-expressing ($Cas^{-/-}$ and Cas) cells, respectively, and error bars show standard deviation. (C) Tyrosine phosphorylation of cellular protein induced by FN attachment. Fifty $\mu$g of cell lysates extracted from Cas-deficient ($Cas^{-/-}$) and Cas-re-expressing ($Cas^{-/-}$ and Cas) at 0, 15, 30, 60, and 120 min after plating were separated by 7.5% SDS-PAGE, blotted to nitrocellulose membrane, and probed with anti-phosphotyrosine antibody, 4G10 [27]. The representative result is shown. The positions of major tyrosine phosphorylated proteins (pp125, pp80, pp70, and pp42) are indicated by arrows and the positions of protein markers are shown on the left.

Cas-re-expressing cells. The morphological changes in Cas-deficient and Cas-re-expressing cells at various periods of time (15, 30, 60, and 120 min) after plating are shown in Fig. 4A and the mean percentages of flattened cells at each time period are shown in Fig. 4B. During the observation periods, the disparity in cell spreading was most apparent at 15 min after plating, when more than 70% of the Cas-re-expressing cells had flattened, while only ~20% of the Cas-deficient cells showed a flattened phenotype. The spreading defect in Cas-deficient cells was still observed at 30 min. At 60 min, although some Cas-deficient cells still remained round and refractile, the morphological appearances of Cas-deficient and Cas-re-expressing cells were almost similar. At 120 min, ~100% of both types of cells have flattened and no obvious differences were observed. These results demonstrated that Cas accelerates cell spreading and enhances cell flattening on FN.

To investigate whether the delayed spreading in Cas-deficient cells might reflect alterations in protein-tyrosine phosphorylation, the cell lysates at 0, 15, 30, 60, and 120 min after plating were subjected to Western blot with anti-phosphotyrosine antibody, 4G10 [27]. As shown in Fig. 4C, FN stimulation rapidly induced tyrosine phosphorylation of similar sets of cellular proteins in both Cas-deficient and Cas-re-expressing cells (Fig. 4C). The major tyrosine phosphorylated proteins were pp125, pp80, pp70, and pp42 (indicated by arrows in Fig. 4C), which are consistent with the results of previous studies [7–9]. Thus, Cas-deficiency did not greatly affect FN-induced protein-tyrosine phosphorylation and it does not seem to be likely that the delayed cell spreading in Cas-deficient cells was due to impaired protein-tyrosine phosphorylation.

## DISCUSSION

In this report, we investigated roles of Cas in cellular function. To confirm any possible defects in Cas-deficient cells were due to Cas-deficiency, we first introduced Cas into Cas-deficient fibroblasts and established Cas-re-expressing fibroblasts. Three independent clones re-expressing Cas at a comparable level to wild-type cells were chosen, which exhibited essentially the same results.

Prior to the functional assays, we examined whether re-expression of Cas restored defects observed in Cas-deficient cells. Cas re-expressing fibroblasts showed normal morphological appearances (Fig. 1B) and also exhibited long actin stress fiber formation (data not shown) that are similar to those observed in wild-type cells. Therefore, re-expression of Cas restored morphological and cytoskeletal abnormalities observed in Cas-deficient cells.

Wound healing and cell migration assays demonstrated that Cas significantly promoted cell movement and cell migration toward FN (Fig. 2 and Fig. 3). Similar findings have been reported using chinese hamster ovary (CHO) cells [31] or using human pancreas carcinoma (FG) cells [29]. Cary *et al.* reported that CHO cells overexpressing FAK exhibited an increased migration ability as compared with the vector-transformed control cells. They showed that the increased level of migration was correlated with the increase in tyrosine phosphorylation of Cas and that expression of a mutant form of Fak, which lacks the binding site of Cas, failed to exhibit the enhanced migratory ability [31]. Subsequently, Klemke *et al.* reported that FG-M cells, which were separated from FG cells as a migration competent subpopulation, exhibited increased tyrosine phosphorylation of Cas as compared with the parent FG cells and that transient expression of Cas in Cos cells increased the cell migratory ability [29]. These reports suggested that expression and tyrosine phosphorylation of Cas are involved in the migratory ability of the cells. However, since these studies were carried out using CHO cells overexpressing Fak, pancreatic carcinoma cells and its subpopulation, or Cos cells transiently expressing Cas, it has remained unclear to what extent these results indeed reflect the physiological roles of Cas. In this respect, we used Cas-deficient and Cas-re-expressing primary fibroblasts and compared their biological properties. Therefore, our findings provide more direct and physiological evidence that Cas promotes cell movement and cell migration.

In cell spreading assay, although Cas-deficient cells and Cas-re-expressing cells similarly attached to the FN-coated dishes, Cas-deficient cells exhibited a significant delay in cell spreading. In addition, protein-tyrosine phosphorylation was almost equally induced in Cas-deficient and Cas-re-expressing cells. Although there remains a possibility that slight but significant tyrosine-phosphorylation of protein(s) could not be detected in our Western blot, it could be strongly suggested that the delayed cell spreading of Cas-deficient cells was, for the most part, due to the impaired actin assembly caused by Cas-deficiency.

It is to be noted that Src-deficient fibroblasts also showed a reduced rate of cell spreading when plated on FN [30]. The phenotypical coincidence between Src-deficient and Cas-deficient fibroblasts strongly suggests that Src and Cas cooperatively function at a critical step for fibroblasts to spread on FN. Interestingly, the impaired spreading of Src-deficient cells was restored by the introduction of only the SH2 and SH3 regions of Src and the kinase domain of Src was dispensable [30]. In addition, studies using mutant cell lines lacking a protein-tyrosine kinase, such as Src, Fak, Fyn, or Abl, revealed that the localization of Cas to focal adhesion and its tyrosine phosphorylation following FN-stimulation were abolished only in Src-deficient cells [32, 33] and expression of a kinase-negative form of Src could rescue these abnormalities [23, 33]. Considering that Cas binds to the SH3 and SH2 regions of Src through its proline-rich region and $Tyr^{762}$, respectively [20], Src can be regarded as a recruiting molecule that accompanies Cas to focal adhesions in a kinase activity-independent mechanism. Therefore, it can be supposed that the defect in spreading on FN in Src-deficient cells seems to be, at least in part, owing to the failure in localization of Cas to focal adhesions.

In summary, we have demonstrated that Cas functions as a molecule promoting cell motility, cell migration, and cell spreading. These findings have clarified specific roles of Cas in cellular function and suggest that Cas would be implicated in various physiological and pathological processes, such as would healing, chemotaxis, and tumor metastasis.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Hynes, R. O. (1992) *Cell* **69,** 11–25.
2. Burridge, K., Fath, K., Kelly, T., Nuckolls, G., and Turner, C. (1988) *Ann. Rev. Cell Biol.* **2,** 487–525.
3. Clark, E. A., and Brugge, J. S. (1995) *Science* **268,** 233–239.
4. Schaller, M. D., Borgman, C. A., and Parsons, J. T. (1993) *Mol. Cell. Biol.* **13,** 785–791.
5. Burridge, K., Turner, C. E., and Romer, L. H. (1992) *J. Cell Biol.* **119,** 893–903.
6. Bockholt, S. M., and Burridge, K. (1995) *Cell Adhes. Commun.* **3,** 91–100.
7. Vuori, K., and Ruoslahti, E. (1995) *J. Biol. Chem.* **270,** 22259–22262.
8. Morino, N., Mimura, T., Hamasaki, K., Tobe, K., Ueki, K., Kikuchi, K., Takehara, K., Kadowaki, T., Yazaki, Y., and Nojima, Y. (1995) *J. Biol. Chem.* **270,** 269–273.
9. Nojima, Y., Morino, N., Mimura, T., Hamasaki, K., Furuya, H., Sakai, R., Sato, T., Tachibana, K., Morimoto, C., Yazaki, Y., and Hirai, H. (1995) *J. Biol. Chem.* **270,** 15398–15402.
10. Kanner, S. B., Reynolds, A. B., Wang, H. C., Vines, R. R., and Parsons, J. T. (1991) *EMBO J.* **10,** 1689–1698.
11. Matsuda, M., Mayer, B. J., Fukui, Y., and Hanafusa, H. (1990) *Science* **248,** 1537–1539.

12. Reynolds, A. B., Kanner, S. B., Wang, H. C., and Parsons, J. T. (1989) *Mol. Cell. Biol.* **9,** 3951–3958.
13. Sakai, R., Iwamatsu, A., Hirano, N., Ogawa, S., Tanaka, T., Mano, H., Yazaki, Y., and Hirai, H. (1994) *EMBO J.* **13,** 3748–3756.
14. Sakai, R., Iwamatsu, A., Hirano, N., Ogawa, S., Tanaka, T., Nishida, J., Yazaki, Y., and Hirai, H. (1994) *J. Biol. Chem.* **269,** 32740–32746.
15. Ingham, R. J., Krebs, D. L., Barbazuk, S. M., Turck, C. W., Hirai, H., Matsuda, M., and Gold, M. R. (1996) *J. Biol. Chem.* **271,** 32306–32314.
16. Schraw, W., and Richmond, A. (1995) *Biochemistry* **34,** 13760–13767.
17. Casamassima, A., and Rozengurt, E. (1997) *J. Biol. Chem.* **272,** 9363–9370.
18. Ojaniemi, M., and Vuori, K. (1997) *J. Biol. Chem.* **272,** 25993–25998.
19. Polte, T. R., and Hanks, S. K. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10678–10682.
20. Nakamoto, T., Sakai, R., Ozawa, K., Yazaki, Y., and Hirai, H. (1996) *J. Biol. Chem.* **271,** 8959–8965.
21. Khwaja, A., Hallberg, B., Warne, P. H., and Downward, J. (1996) *Oncogene* **12,** 2491–2498.
22. Salgia, R., Pisick, E., Sattler, M., Li, J. L., Uemura, N., Wong, W. K., Burky, S. A., Hirai, H., Chen, L. B., and Griffin, J. D. (1996) *J. Biol. Chem.* **271,** 25198–25203.
23. Schlaepfer, D. D., Broome, M. A., and Hunter, T. (1997) *Mol. Cell. Biol.* **17,** 1702–1713.
24. Liu, F., Hill, D. E., and Chernoff, J. (1996) *J. Biol. Chem.* **271,** 31290–31295.
25. Garton, A. J., Flint, A. J., and Tonks, N. K. (1996) *Mol. Cell. Biol.* **16,** 6408–6418.
26. Honda, H., Oda, H., Nakamoto, T., Honda, Z.-i., Sakai, R., Suzuki, T., Saito, T., Nakamura, K., Nakao, K., Ishikawa, T., Katsuki, M., Yazaki, Y., and Hirai, H. (1998) *Nat. Genet.* **19,** 361–365.
27. Morrison, D. K., Kaplan, D. R., Rhee, S. G., and Williams, L. T. (1990) *Mol. Cell. Biol.* **10,** 2359–2366.
28. Xu, W., Baribault, H., and Adamson, E. D. (1998) *Development* **125,** 327–337.
29. Klemke, R. L., Leng, J., Molander, R., Brooks, P. C., Vuori, K., and Cheresh, D. A. (1998) *J. Cell. Biol.* **140,** 961–972.
30. Kaplan, K. B., Swedlow, J. R., Morgan, D. O., and Varmus, H. E. (1995) *Genes Dev.* **9,** 1505–1517.
31. Cary, L. A., Han, D. C., Polte, T. R., Hanks, S. T., and Guan, J.-L. (1998) *J. Cell Biol.* **140,** 211–221.
32. Hamasaki, K., Mimura, T., Morino, N., Furuya, H., Nakamoto, T., Aizawa, S., Morimoto, C., Yazaki, Y., Hirai, H., and Nojima, Y. (1996) *Biochem. Biophys. Res. Commun.* **222,** 338–343.
33. Nakamoto, T., Sakai, R., Honda, H., Ogawa, S., Ueno, H., Suzuki, T., Aizawa, S., Yazaki, Y., and Hirai, H. (1997) *Mol. Cell. Biol.* **17,** 3884–3897.