v-Crk regulates membrane dynamics and Rac activation

Myeong Gu Yeo and Woo Keun Song*

Cell Dynamics Research Center and Bioimaging Research Center, Department of Life Science, Gwangju Institute of Science and Technology; Buk-gu, Gwangju, Korea

Key words: v-Crk, Rac, lamellipodia dynamics, cell migration, p130CAS

Cell migration is an integrated process that involves cell adhesion, protrusion and contraction. We recently used CAS (Crk-associated substrate, 130CAS)-deficient mouse embryo fibroblasts (MEFs) to examine contribution made to v-Crk to that process via its interaction with Rac1. v-Crk, the oncogenic product of avian sarcoma virus CT10, directly affects membrane ruffle formation and is associated with Rac1 activation, even in the absence of CAS, a major substrate for Crk. In CAS-deficient MEFs, cell spreading and lamellipodium dynamics are delayed; moreover, Rac activation is significantly reduced and it is no longer targeted to the membrane. However, expression of v-Crk by CAS-deficient MEFs increased cell spreading and active lamellipodium protrusion and retraction. v-Crk expression appears to induce Rac1 activation and its targeting to the membrane, which directly affects membrane dynamics and, in turn, cell migration. It thus appears that v-Crk/Rac1 signaling contributes to the regulation of membrane dynamics and cell migration, and that v-Crk is an effector molecule for Rac1 activation that regulates cell motility.

Cell migration is a central event in a wide array of biological and pathological processes, including embryonic development, inflammatory responses, angiogenesis, tissue repair and regeneration, cancer invasion and metastasis, osteoporosis and immunological responses. Although the molecular basis of cell migration has been studied extensively, the underlying mechanisms are still not fully understood. It is known that cell migration is an integrated process that involves formation of cell adhesions and/or cell polarization, membrane protrusion in the direction of migration (e.g., filopodium formation and lamellipodium extension), cell body contraction and tail detachment. Formation of cell adhesions, including focal adhesions, fibrillar adhesions and podosomes are the first step in cell migration. Cell adhesions are stabilized by attachment to the extracellular matrix (ECM) mediated by integrin transmembrane receptors, which are also linked to various cytoplasmic proteins and the actin cytoskeleton, which provide the mechanical force necessary for migration. The next steps in the process of cell migration are filopodium formation and lamellipodium extension. These are accompanied by actin polymerization and microtubule dynamics, which also contribute to the control of cell adhesion and migration.

Focal adhesions are highly dynamic structures that form at sites of membrane contact with the ECM and involve the activities of several cellular proteins, including vinculin, focal adhesion kinase (FAK), Src family kinase, paxillin, CAS (CAS-associated substrate, p130CAS) and Crk. A deficiency in focal adhesion protein is associated with the severe defects in cell motility and results in embryonic death. For example, FAK−/−embryos disrupt mesoderm development in mice and delays cell migration in vitro, which reflects impaired assembly and disassembly of the focal adhesions. In addition, mouse embryonic fibroblasts (MEFs) lacking Src kinase showed a reduced rate of cell spreading that resulted in embryonic death. Taken together, these findings strongly support the idea that cell adhesion complexes play crucial roles in cell migration.

CAS is a hyperphosphorylated protein known to be a major component of focal adhesion complexes and to be involved in the transformation of cells expressing v-Src or v-Crk. CAS-deficient mouse embryos die in utero and show marked systematic congestion and growth retardation, while MEFs lacking CAS show severely impaired formation and bundling of actin stress fibers and delayed cell motility. Conversely, transient expression of CAS in COS7 cells increases cell migration. Crk-null mice also exhibit lethal defects in embryonic development, which is consistent with the fact that CAS is a major substrate for v-Crk, and both CAS and v-Crk are necessary for induction of cell migration. v-Crk consists of a viral gag sequence fused to cellular Crk sequences, which contain Src homology 2 (SH2) and SH3 domains but no kinase domain, and both CAS and paxillin bind to SH2 domains. Despite the absence of a kinase domain, cell expressing v-Crk show upregulation of tyrosine phosphorylation of CAS, FAK and paxillin, which is consistent with v-Crk functioning as an adaptor protein. Moreover, this upregulation of tyrosine phosphorylation correlates well with the transforming activity of v-Crk. By contrast, tyrosine phosphorylation of FAK and CAS is diminished in Src kinase-deficient cells expressing v-Crk, and they are not targeted to the membrane, suggesting v-Crk signaling is Src kinase-dependent. After formation of the CAS/v-Crk complex, v-Crk likely transduces cellular signaling to Src kinase and FAK. Notably, tyrosine phosphorylation of FAK and cell migration and spreading are all enhanced when v-Crk is introduced into CAS-deficient MEFs. We therefore suggest that v-Crk activity, but not cellular Crk activity, during cell migration and spreading is CAS-independent.
Membrane dynamics such as lamellipodium protrusion and membrane ruffling reportedly involve Rac1, αβ1 integrin, Arp2/3, and N-WASP, and are enhanced in v-Crk-expressing CAS-deficient MEFs. Moreover, expression in those cells of N17Rac1, a dominant defective Rac1 mutant, abolished membrane dynamics at early times and delayed cell migration. v-Crk-expressing, CAS-deficient MEFs transfected with N17Rac1 did not begin spreading until one hour after being plated on fibronectin, and blocking Rac activity suppressed both membrane dynamics and cell migration. We therefore suggest that v-Crk is involved in cell attachment and spreading, and that this process is mediated by Rac1 activation. In addition, v-Crk expression apparently restores lamellipodium formation and ruffle retraction in CAS-deficient MEFs. Thus v-Crk appears to participate in a variety cellular signaling pathways leading to cell spreading, Rac1 activation, membrane ruffling and cell migration, even in the absence of CAS, its major substrate protein.

In fibroblasts, the Rho family of small GTP-binding proteins (e.g., Cdc42, Rac and Rho) functions to control actin cytoskeleton turnover, including filopodium extension, lamellipodium formation and generation of actin stress fibers and focal adhesions. These GTPases function in a cascade, such that activation of Cdc42 stimulation and generation of actin stress fibers and focal adhesions.22 Once activated, Rho controls cell migration. Cell adhesion to ECM leads to the translocation of Rac1 and Cdc42 from the cytosol to the plasma membrane, where they regulate actin polymerization at the leading edge. Dominant negative Rac and Cdc42 mutants inhibit the signaling to cell spreading initiated by the interaction of integrin with ECM. The fact that cellular levels of activated Rac are higher in cells adhering to ECM than in suspended cells further suggests that activation of Rac and Cdc42 is a critical step leading to membrane protrusion and ruffle formation. It is noteworthy in this regard that v-Crk is able to induce Rac activation and its translocation to plasma membrane.

Overall, the findings summarized in this article demonstrate that v-Crk participates in several steps leading to cell adhesion and spreading (Fig. 1), and the targeting of v-Crk to focal adhesion sites appears to be a prerequisite for regulation of cell migration and spreading via Rac activation. To fully understand its function, however, it will be necessary to clarify the role of v-Crk in Rac1 and Cdc42 activation initiated by integrin-ECM interactions.

Acknowledgements

This study was supported by grants from the Cell Dynamics Research Center (R11-2007-007-02001-0), the Bioimaging Research Center and the Research Program for New Drug Target Discovery (2006-02594, The Ministry of Science and Technology, South Korea).

References

1. Webb DJ, Parsons JT, Horwitz AF. Adhesion assembly, disassembly and turnover in migrating cells—over and over and over again. Nat Cell Biol 2002; 4:97-100.
2. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Horwitz G, Parsons JT, Horwitz AR. Cell Migration: Integrating Signals from Front to Back 2003; 1704-9.
3. Lauffenburger DA, Horwitz AF. Cell Migration: A Physically Integrated Molecular Process. Cell 1996; 84:359-69.
4. Honda H, Oda H, Nakamoto T, Honda ZI, Sakai R, Suzuki T, Saito T, Nakamura K, Nakao K, Ishikawa T, Ikutsu M, Yatsuka Y, Hirai H, Cardiovascular abnormality, impaired actin bundling and resistance to Src-induced transformation in mice lacking pl30Cas. Nat Genet 1998; 19:361-5.
5. Owen EA, Preedy FJ, Thomas K, Vicente-Manzaneque M, Ray BJ, Horwitz AF, Parsons JT, Beggs HE, Stanley ER, Bouton AH. Regulation of lamellipodial persistence, adhesion turnover, and motility in macrophages by focal adhesion kinase 2007; 1275-87.
6. Craig SW, Chen H. Lamellipodia Proliferation: Moving Interactions of Vinculin and Ap 2003/1: 13:236-8.
7. Ilc D, Furuta T, Kanazawa S, Takeda N, Sobe K, Nakatsuji N, Nomura S, Fujimoto J, Okada M, Yamamoto T, Aizawa-Shinichi. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 1995; 377:539-44.
8. Yeo MG, Partridge MD, Erzarry EJ, Shen Q, Gundersen GG, Marcanzon EE. Src SH2 Arginine 175 Is Required for Cell Motility: Specific Focal Adhesion Kinase Targeting and Focal Adhesion Assembly Function 2006; 4399-409.
9. Klinghoffer RA, Sachsenmaier C, Cooper JA, Soriano P. Src family kinases are required for integrin but not PDGFR signal transduction. The EMBO Journal 1999; 18:2459-71.
10. Matsuda M, Mayer BJ, Fukui Y, Hanafusa H. Binding of transforming protein, P47gag-crk, to a broad range of phosphotyrosine-containing proteins. Science 1998; 248:1537-9.
11. Huang J, Hamasaki H, Nakamoto T, Honda H, Hirai H, Saito M, Takato T, Sakai R. Differential Regulation of Cell Migration, Actin Stress Fiber Organization, and Cell Transformation by Functional Domains of Crk-associated Substrate. J Biol Chem 2002; 277:27265-72.
12. Yeo MG, Sung BH, Oh HJ, Park ZY, Marcanzon EE, Song WK. Focal adhesion targeting of v-Crk is essential for FAK phosphorylation and cell migration in mouse embryo fibroblasts deficient of src family kinase protein p130Cas. Journal of Cellular Physiology 2008; 214:664-13.
13. Park TJ, Boyd K, Curran T. Cardiovascular and Craniofacial Defects in Crk-Null Mice. Mol Cell Biol 2006; 26:6272-82.
14. Klemke RL, Leng J, Molander R, Brooks PC, Vuori K, Cheresh DA. CAS/Crk Coupling Serves as a “Molecular Switch” for Induction of Cell Migration. J Cell Biol 1998; 140:961-72.
15. Mayer BJ, Hamaguchi M, Hanafusa H. A novel viral oncogene with structural similarity to phospholipase C. Nature 1988; 332:272-5.
16. Nievers MG, Birge RB, Greulich H, Verkleij AJ, Hanafusa H, van Bergen en Henegouwen PM. v-Crk-induced cell transformation: changes in focal adhesion composition and signaling. J Cell Sci 1997; 110:389-99.

17. Sakai R, Hirano N, Ogawa S, Tanaka T, Mano H, Yazaki Y, Hirai H. A novel signaling molecule, p130, forms stable complexes in vivo with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner. EMBO 1994; 13:3748-56.

18. Allen WE, Jones GE, Pollard JW, Ridley AJ. Rho, Rac and Cdc42 regulate actin organization and cell adhesion in macrophages. J Cell Sci 1997; 110:707-20.

19. Pinco KA, He W, Yang JT. alphavbeta1 Integrin Regulates Lamellipodia Protrusion via a Focal Complex/Focal Adhesion-independent Mechanism. Mol Biol Cell 2002; 13:3203-17.

20. Legg JA, Bompad G, Dawson J, Morris HL, Andrew N, Cooper L, Johnston SA, Tramontanis G, Macheshy LM. N-WASP Involvement in Dorsal Ruffle Formation in Mouse Embryonic Fibroblasts. Mol Biol Cell 2007; 18:678-87.

21. Sung BH, Yeo MG, Oh HJ, Song WK. v-Crk Induces Rac-dependent Membrane Ruffling and Cell Migration in CAS-deficient Embryonic Fibroblasts. Molecules and cells 2008; 25:131-7.

22. Hildebrand JD, Taylor JM, Parsons JT. An SH3 domain-containing GTPase-activating protein for Rho and Cdc42 associates with focal adhesion kinase. Mol Cell Biol 1996; 16:3169-78.

23. Del Pozo MA, Kisses WB, Alderson NB, Meller N, Hahn KM, Schwartz MA. Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. Nat Cell Biol 2002; 4:232-9.

24. Price LS, Leng J, Schwartz MA, Bokoch GM. Activation of Rac and Cdc42 by Integrins Mediates Cell Spreading. Mol Biol Cell 1998; 9:1863-71.