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The Diaphanous-related Formin FHOD1 Associates with ROCK1 and Promotes Src-dependent Plasma Membrane Blebbing*[S]

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Diaphanous-related formins (DRFs) mediate GTPase-triggered actin rearrangements to regulate central cellular processes, such as cell motility and cytokinesis. The DRF FHOD1 interacts with the Rho-GTPase Rac1 and mediates formation of actin stress fibers in its deregulated form; the physiologically relevant activities and molecular mechanisms of endogenous FHOD1, however, are still unknown. Here we report that FHOD1 physically associates via the N-terminal part of its FH2 domain with the central domain of ROCK1. Although FHOD1 does not affect the kinase activity of ROCK1, the DRF is an efficient substrate for phosphorylation by ROCK1. Co-expression of FHOD1 and ROCK1 results in the generation of nonapoptotic plasma membrane (PM) blebs, to which the DRF is efficiently recruited. Blebbing induced by FHOD1 and ROCK1 depends on F-actin integrity, the Rho-ROCK cascade, and Src activity and is reminiscent of the recently described PM blebs triggered by expression of Src homology 4 (SH4) domain PM targeting signals. Consistently, endogenous FHOD1 is required in SH4 domain expressing cells for efficient PM blebbing and rounded cell morphology in two-dimensional cultures or three-dimensional matrices, respectively. Efficient association of FHOD1 with ROCK1, as well as recruitment of the DRF to blebs, depends on Src activity, suggesting that the functional interaction between both proteins is regulated by Src. These results define a role for endogenous FHOD1 in SH4 domain-induced blebbing and suggest that its activity is regulated by ROCK1 in a Src-dependent manner.

In response to intra- and extracellular cues, remodeling of the submembranous cytoskeleton constantly adjusts the plasma membrane (PM)[2] of eukaryotic cells. These cytoskeletal reorganizations are primarily controlled by small Rho-GTPases and their downstream signaling cascades, resulting in distinct types of invaginations or protrusions, depending on the specific set of GTPases and effectors involved. In addition to well described PM protrusions, such as lamellipodia and filopodia (1), under certain conditions, cells display on their surface highly dynamic rounded structures referred to as PM blebs (2). PM blebbing results from local destabilization of the cortical actin meshwork that causes expansion of the PM due to the osmotic pressure of the cell interior. Following this expansion phase, blebs typically briefly remain static before local actin polymerization and actin-myosin contraction events are thought to guide retraction of the bleb (3–7). PM blebbing has long been observed as an early event in apoptotic and necrotic processes (8–10). More recently, nonapoptotic PM blebs were identified to play roles in distinct cellular processes, such as cytokine release, cytokinesis, embryonic stem cell motility, or cancer cell invasion (11–14). Although PM blebbing seems to follow the common overall scheme of expansion and retraction, mechanistic differences exist between distinct types of nonapoptotic PM blebs, in particular in regard to the stimulus that initiates blebbing. Typically, blebbing is induced in a three-dimensional environment by yet to be identified stimuli and is thought to facilitate directed cell movement of, for example, tumor or germ cells (11, 14–16). More amenable for molecular analysis, several model systems have been described in which cell blebbing is efficiently observed under two-dimensional cell culture conditions. Deficiency in the actin-binding protein filamin A (5, 6) or the tumor suppressor p53 (17) as well as expression of the Dia-interacting protein DIP (18) or an effector loop mutant of active Rac1 (19) can cause efficient PM blebbing. We recently reported that expression of SH4 membrane-targeting domains, corresponding to an 18-aa short peptide with N-terminal acylation that mediates PM targeting of, for example, Src

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[2] The abbreviations used are: PM, plasma membrane; DRF, Diaphanous-related formin; FH1, -2, and -3, formin homology 1, 2, and 3, respectively; SH4, Src homology 4; aa, amino acid(s); CHO, Chinese hamster ovary; TRITC, tetramethylrhodamine isothiocyanate; HA, hemagglutinin; WT, wild type; IP, immunoprecipitation; RNAi, RNA interference; GFP, green fluorescent protein; MLC, myosin light chain; DAD, Diaphanous autoregulatory domain.

Fackler, O. T. (2005) *Exp. Cell Res.* **306,** 192–202
31. Koka, S., Neudauer, C. L., Li, X., Lewis, R. E., McCarthy, J. B., and Westendorf, J. J. (2003) *J. Cell Sci.* **116,** 1745–1755
32. Takeya, R., and Sumimoto, H. (2003) *J. Cell Sci.* **116,** 4567–4575
33. Madrid, R., Gasteier, J. E., Bouchet, J., Schroder, S., Geyer, M., Benichou, S., and Fackler, O. T. (2005) *FEBS Lett.* **579,** 441–448
34. Schonichen, A., Alexander, M., Gasteier, J. E., Cuesta, F. E., Fackler, O. T., and Geyer, M. (2006) *J. Biol. Chem.* **281,** 5084–5093
35. Takeya, R., Taniguchi, K., Narumiya, S., and Sumimoto, H. (2008) *EMBO J.* **27,** 618–628
36. Wang, Y., El-Zaru, M. R., Surks, H. K., and Mendelsohn, M. E. (2004) *J. Biol. Chem.* **269,** 24420–24426
37. Klinghoffer, R. A., Sachsenmaier, C., Cooper, J. A., and Soriano, P. (1999) *EMBO J.* **18,** 2459–2471
38. Peytavi, R., Hong, S. S., Gay, B., d'Angeac, A. D., Selig, L., Benichou, S., Benarous, R., and Boulanger, P. (1999) *J. Biol. Chem.* **274,** 1635–1645
39. Rauch, S., Pulkkinen, K., Saksela, K., and Fackler, O. T. (2008) *J. Virol.* **82,** 2818–2829
40. Haller, C., Rauch, S., and Fackler, O. T. (2007) *PLoS ONE* **2,** e1212
41. Benichou, S., Bomsel, M., Bodeus, M., Durand, H., Doute, M., Letourneur, F., Camonis, J., and Benarous, R. (1994) *J. Biol. Chem.* **269,** 30073–30076
42. Sahai, E. (2005) *Curr. Opin. Genet. Dev.* **15,** 87–96
43. Pinner, S., and Sahai, E. (2008) *Nat. Cell Biol.* **10,** 127–137
44. Tominaga, T., Sahai, E., Chardin, P., McCormick, F., Courtneidge, S. A., and Alberts, A. S. (2000) *Mol. Cell* **5,** 13–25
45. Gasman, S., Kalaidzidis, Y., and Zerial, M. (2003) *Nat. Cell Biol.* **5,** 195–204
46. Koka, S., Minick, G. T., Zhou, Y., Westendorf, J. J., and Boehm, M. B. (2005) *Biochem. Biophys. Res. Commun.* **336,** 1285–1291
47. Aspenstrom, P., Richnau, N., and Johansson, A. S. (2006) *Exp. Cell Res.* **312,** 2180–2194
48. Harris, E. S., Rouiller, I., Hanein, D., and Higgs, H. N. (2006) *J. Biol. Chem.* **281,** 14383–14392
49. Wolf, K., Mazo, I., Leung, H., Engelke, K., von Andrian, U. H., Deryugina, E. I., Strongin, A. Y., Brocker, E. B., and Friedl, P. (2003) *J. Cell Biol.* **160,** 267–277