Tight control and regulation of cytoskeletal rearrangements is one of the great challenges that a cell has to face in the establishment of fundamental cellular processes such as cell motility, cytokinesis, and morphogenesis. Over the past few years, the family of formins has emerged as a diverse group of multidomain proteins that contains a unique actin binding formin homology 2 (FH2) domain. Catalytic activity of the FH2 domain intertwines these proteins with different aspects of actin cytoskeleton dynamics and the consequent transmission of extracellular signals to transcriptional programs driven mainly by the serum response factor (SRF) (Faix and Grosse, 2006). By forming a tethered dimer, the FH2 domains promote barbed end actin polymerization (Otomo et al., 2005b; Xu et al., 2004) and may also lead to the formation of higher order actin structures through the bundling of preexisting filaments (Harris et al., 2006).

FHOD1 belongs to the subfamily of Diaphanous-related formins (DRFs), which have been shown to act as effectors of GTPases of the Rho family. DRFs are characterized by the formation of a basal state of autoinhibition, which is achieved by intramolecular interaction between the C-terminal Diaphanous autoregulatory domain (DAD) and the FH3 domain, which, in cooperation with a GTPase binding domain (GBD), forms an N-terminal array of regulatory sequences (Figure 1A). In the case of a prototypic formin, mDia1, structural and functional evidence exists that autoinhibition can be relieved after binding of an active Rho GTPase to the GBD (Brandt et al., 2007; Lammers et al., 2005; Otomo et al., 2005a), whereas additional signals may be required for full activity of some DRFs. Further characterization of DRF specificity for different Rho GTPases has led to the delineation of specific subsets of DRF/GTPase modules, underlining the fundamental role of the GBD architecture in maintaining a well-defined balance in the complexity of formin-mediated signaling events.

Previous structural analysis of the FH2 domain of yeast Bni1p (Otomo et al., 2005b; Xu et al., 2004), as well as structure determinations of the mDia1 N-terminal regulatory region, (Lammers et al., 2005; Nezami et al., 2006; Otomo et al., 2005a) have led to decisive progress in the field and significantly affected our present understanding of formin function and their sophisticated regulation. However, based on low sequence similarities, it is imminent that the structure and function of the various Diaphanous-related formins cannot be generalized and structure predictions so far often fail to decipher the GBD and FH3 domains in given DRFs.

So how do you find a GBD that is hiding away? Schulte et al. (2008) set out to shed some light on this subject by solving the crystal structure of the N terminus of mammalian FHOD1, which was a previously unknown region. Unexpectedly, they identified two domains with a GBD that is structurally unrelated to the common GBDs of formins. Very much in line, however, with the domain architecture and structure of the prototypic formin mDia1 is the adjacent FH3 domain of FHOD1 that is composed of five armadillo repeats that provide the surface for intramolecular interaction with the DAD (Figure 1B). Using domain superimposition with the mDia1 FH3 domain, they could identify a valine at position 228 that, when mutated to glutamate, would disrupt autoinhibition as analyzed by functional assays. However, a real surprise was around the corner when they took a closer look at the domain N-terminal of the armadillo repeats. Schulte et al. (2008) found that this domain contains a β sheet that reveals an ubiquitin superfold very similar to that of the Ras-binding domain of c-Raf1. This suggested to them that they were looking at the GBD of FHOD1. Interestingly, the deletion of the GBD did not result in a constitutive active mutant, as implicated by other studies of DRFs. In fact, in the case of FHOD1, its cellular biological activity was lost in mutants lacking the GBD, suggesting that this domain is essential for its effects on actin assembly in vivo. Nevertheless, this domain is indeed a GBD, as directly demonstrated by activated Ras using isothermal titration calorimetry (ITC) experiments. In addition, by screening 33 GTPases of the Ras superfamily for their ability to recruit FHOD1 to the cell cortex, the authors found that only Rac efficiently relocalized FHOD1, although some activity with Ras was also detected. Thus, the formin FHOD1 may be involved in Rac signaling as expected from previous studies (Westendorf, 2001) but also appears as a candidate effector molecule for Ras.

Several interesting questions obviously arise from these findings. What has Ras got to do with it? As mentioned earlier, formins are potent regulators of actin filament assembly, functions that have not been directly linked with Ras. Ras is well known for its effects on cell proliferation and transformation. Interestingly, these functions require a balanced crosstalk between Ras and Rho signaling. An interesting possibility is that FHOD1 integrates aspects of these pathways.

Another central question remains: how does the recruitment through Rac-GTPases, proteins that are involved in cell spreading and lamellipodia formation, fit with the counteractive biological activity of FHOD1 exerted on stress fibers and actin bundling, which involves the recently reported phosphorylation-activation mechanism by Rho kinase (ROCK) (Hannemann et al., 2008; Takeya et al., 2008)? Could FHOD1 function as a scavenger for active Rac, while being recruited to them that they were looking at the GBD of FHOD1. Interestingly, the deletion of the GBD did not result in a constitutive active mutant, as implicated by other studies of DRFs. In fact, in the case of FHOD1, its cellular biological activity was lost in mutants lacking the GBD, suggesting that this domain is essential for its effects on actin assembly in vivo. Nevertheless, this domain is indeed a GBD, as directly demonstrated by activated Ras using isothermal titration calorimetry (ITC) experiments. In addition, by screening 33 GTPases of the Ras superfamily for their ability to recruit FHOD1 to the cell cortex, the authors found that only Rac efficiently relocalized FHOD1, although some activity with Ras was also detected. Thus, the formin FHOD1 may be involved in Rac signaling as expected from previous studies (Westendorf, 2001) but also appears as a candidate effector molecule for Ras.

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to the plasma membrane for phosphorylation through ROCK? In such a scenario, localized Rho/ROCK activity may be fine-tuned or amplified by simultaneous inhibition of Rac. Clearly, FHOD1 does not fit our current view of formin regulation, suggesting that, with over 14 formins known in humans, there are many more surprises to be unveiled. It is thus becoming increasingly evident that many formins may exert important functions beyond their actin-assembling properties.

REFERENCES


