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The Diaphanous-related formin dDia1 is required for highly directional phototaxis and formation of properly sized fruiting bodies in *Dictyostelium*

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ABSTRACT

Diaphanous-related formins (DRFs) act as downstream effectors of Rho family GTPases and drive the formation and elongation of linear actin filaments in various cellular processes. Here we analyzed the DRF dDia1 from *Dictyostelium* cells. The biochemical characterization of recombinant dDia1-FH1FH2 by bulk polymerization assays and single filament TIRF microscopy revealed that dDia1 is a rather weak nucleator. Addition of any of the three *Dictyostelium* profilin isoforms, however, markedly accelerated formin-mediated actin filament barbed end elongation in TIRF assays. Interestingly, filament elongation was significantly faster in presence of DdPFN I (profilin I) when compared to the other two isoforms, suggesting selectivity of dDia1 for DdPFN I. Additionally, we frequently observed dissociation of the formin from growing barbed ends. These findings are consistent with dilution-induced depolymerization assays in presence of dDia1-FH1FH2 showing that dDia1 is a weak capper in comparison with heterodimeric capping protein. To study the physiological role of this formin, we created cell lines lacking dDia1 or overexpressing GFP-tagged dDia1. Of note, constitutively active dDia1 accumulated homogenously in the entire pseudopod suggesting that it controls microfilament architecture to regulate cell migration. Comparison of wild type and dDia1-null cells in random cell migration and chemotaxis toward a cAMP gradient revealed no major differences. By contrast, phototaxis of dDia1-deficient cells during the multicellular stage was markedly impaired. While wild type slugs moved with high directionality toward the light source, the trails of dDia1-null slugs displayed a characteristic V-shaped profile and deviated in angles between 50° and 60° from the path of the incident light. Possibly in conjunction with this defect, dDia1-null cells also formed substantially smaller fruiting bodies. These findings demonstrate dDia1 to be critically involved in collective cell migration during terminal differentiation.

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Introduction

Formins are large multi-domain proteins that catalyze *de novo* nucleation and elongation of linear actin filaments and are involved in the assembly of various structures such as filopodia, stress fibers and actin cables (Faix and Grosse, 2006; Chesarone et al., 2010). The signature sequence of formins is the formin homology-2 (FH2) domain (Goode and Eck, 2007). This region of about 400 amino acid residues and an adjacent linker sequence form a flexible anti-parallel dimer with a donut-shaped structure that is necessary and sufficient to initiate actin filament assembly (Kovar et al., 2003; Xu et al., 2004). The nucleation activity is most likely mediated by stabilization of highly unstable polymerization intermediates such as actin dimers or trimers by the FH2 domain (Pring et al., 2003; Otomo et al., 2005). Subsequently, the FH2-dimer remains tightly associated with the barbed end and typically slows down filament elongation (Kovar and Pollard, 2004; Harris et al., 2004). To explain this behavior, it has been proposed that the FH2 dimer oscillates between an open and closed conformational state either allowing or preventing addition of new actin subunits onto filament barbed ends (Vavylonis et al., 2006). The FH1 domain, located N-terminally of the FH2 region, comprises several consecutive stretches of poly-L-proline which act as low affinity binding sites for profilin-actin complexes to ensure recruitment and transfer of ATP-G-actin to the catalytic domain thereby markedly increasing the rate of processive actin filament elongation (Kovar et al., 2006; Block et al., 2012). Consistently, previous work revealed that the rate of barbed end growth increases with the number of poly-L-proline stretches within the

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FH1 domain supporting the notion that multiple profilin binding sites promote faster actin assembly by increasing the local actin concentration (Paul and Pollard, 2008). As previously exemplified with vertebrate mDia1 and FMNL3 (Watanabe et al., 1999; Harris et al., 2010), formins seem to strictly depend on the recruitment of profilin-actin complexes by the FH1-domain for actin assembly *in vivo*.

A subset of formins denoted Diaphanous-related formins (DRFs) act as effectors of Rho family GTPases (Watanabe et al., 1997). In these formins, the FH1 and FH2 domains are flanked by an array of regulatory domains at the N-terminus and by a Diaphanous-autoregulatory domain (DAD) at the C-terminus (Alberts, 2001). As opposed to the DAD, which consists of only a small stretch of amino acid residues, the N-terminal regulatory region is considerably larger and includes a GTPase-binding domain (GBD) followed by an adjacent Diaphanous-inhibitory domain (DID) and a dimerization domain (DD) (Li and Higgs, 2005; Rose et al., 2005). The intramolecular interaction of the DID with the DAD leads to autoinhibition, which is released by binding of active Rho-GTPases to the GBD (Watanabe et al., 1999; Alberts, 2001).

The social amoeba *Dictyostelium discoideum* represents an attractive model organism, since it is easy to cultivate and amenable to a variety of molecular manipulation techniques including generation of genetic knockouts (Faix et al., 2004; Linkner et al., 2012). *Dictyostelium* cells endow a complex actin cytoskeleton machinery that allows a cell motility similar to leukocytes (Jin et al., 2009). Moreover, the cells are capable of differentiating into complex multicellular collectives (slugs) containing different cell types. These slugs migrate thermotactically or phototactically to reach environments with improved conditions for the formation of fruiting bodies (Williams, 2010).

D. discoideum cells express 10 different formins (ForA-ForJ), eight of which display the domain architecture of vertebrate DRFs (Rivero et al., 2005). As yet, only ForH/dDia2 and ForC have been characterized in detail. dDia2 has been shown to surf on distal tips of actin filaments in filopodia as they elongated and to be critical for filopodium formation (Schirenbeck et al., 2005). ForC is an unusual formin since its lacks an FH1 region and additionally contains an untypical GBD (Kitayama and Uyeda, 2003; Dames et al., 2011). Consistent with lack of the FH1 region, ForC is devoid of canonical formin activity, but induces the formation of actin filament bundles with mixed polarity (Junemann et al., 2013). Mutants lacking ForC are severely impaired in multicellular development (Kitayama and Uyeda, 2003).

Even though most formins seem to nucleate and elongate actin filaments by a similar mechanism, their individual activities can be highly variable. Moreover, some formins exert additional and unusual activities such as severing or bundling of filaments (Harris et al., 2004, 2006; Chhabra and Higgs, 2006; Schönichen et al., 2013; Jaiswal et al., 2013; Junemann et al., 2013). It is therefore mandatory to determine the specific activities of each formin. In this work we analyzed the biochemical properties and physiological functions of ForF/dDia1. We show that dDia1 is an autoinhibited formin capable of nucleating and processively elongating actin filaments, albeit its nucleation activity is rather low. In cells, dDia1 co-localizes with filamentous actin in the entire pseudopod. Mutants lacking dDia1 show a strikingly aberrant phototaxis and develop smaller fruiting bodies.

Materials and methods

Cell culture, cell lines and transformation of D. discoideum cells

Cells of *D. discoideum* AX2 wild-type strain and of transformants were cultivated in HL5c medium with glucose (Formedium) at 23 °C on polystyrene culture dishes or in shaken suspension as described previously (Junemann et al., 2013). To induce development, the cells were starved in 17 mM Na/K-phosphate buffer, pH 6.0 (PB) and shaken at 150 rpm or plated on PB-agar plates. Cells were transformed by electroporation according to Linkner et al. (2012).

Transformation vectors

For construction of the dDia1 gene (DictyBase ID: DDB0231185) targeting vector, a 5′ BamHI/PstI fragment and a 3′ HindIII/SalI fragment were amplified from genomic AX2 WT DNA by PCR. The primers used for the 5′ fragment were 5′-CGCGGATCCCAATTCCAATAGTAAAAAGTC-3′ and 5′-GCGCTGCAGGATAGTAAGTGCACTCTTACTG-3′ and the primers for the 3′ fragment were 5′-GCGAAGCTTGTCAAGATGATTACAGTGCTA-3′ and 5′-CGCGTCGACCTTTGATCTGCCTGGCTCTTTG-3′. Both fragments were gel purified after cleavage with BamHI/PstI and HindIII/SalI, and cloned into the corresponding sites of pLPBLP containing a Blasticidin S resistance cassette (Faix et al., 2004). The resulting vector was cleaved with BamHI and SalI and used to disrupt the dDia1 gene in WT cells. Null mutants were screened by PCR as described previously (Linkner et al., 2012). For expression of GFP-tagged dDia1 lacking its C-terminal DAD-region dDia1 cDNA was amplified from a λgt11 cDNA library using primers dDia1-BU 5′-CGCGGATCCCAATTCCAATAGTAAAAAGTC-3′ and dDia1-SD 5′-CGCGTCGACTTAACCACCATCACCAACAGAAGTTGAACT-3′, cleaved with BamHI and SalI and inserted into the same site pDGFP-MCS-Neo (Dumontier et al., 2000).

Protein purification

dDia1 coding sequences were amplified from dDia1ΔDAD construct in pDGFP-Neo or from cDNA as BamHI/SalI fragments and cloned into pGEX 6P1 expression vector (GE Healthcare). The following dDia1 constructs were used for expression: dDia1-P2 (amino acids 616–1087), dDia1-P2C (amino acids 616–1220), dDia1-P3 (amino acids 603–1087), dDia1-FH2 (aa 637–1104) and dDia1-N (amino acids 1–576). For expression of N-terminal SNAP-tagged dDia1-P2, the coding sequence of the SNAP tag (Keppler et al., 2003) was obtained by reverse translation using Gene Designer 2.0 software (DNA 2.0 Inc.) for optimal expression in *E. coli*. The designed sequence was synthesized as BglII/BamHI fragment by Genart (Life Technologies), and ligated in sense orientation into the single BamHI site of pGEX-6P-1 (GE Healthcare, USA) to yield expression vector pGEX-6P-1-SNAP. Subsequently, the fragment encoding dDia2-P2 was inserted as BamHI/SalI fragments into pGEX-6P-1-SNAP. The vectors were transformed in *E. coli* strain Rosetta DE3 and expression of GST-tagged constructs was induced with 0.7 mM IPTG at 20–22 °C for 12–16 h. Afterwards, the proteins were purified from bacterial lysates using glutathione agarose 4B (Macherey-Nagel) according to standard protocols. The GST-tag was subsequently cleaved off by addition of PreScission protease (GE Healthcare) and removed by size exclusion chromatography using a preparative Superdex 26/60 S200 column (GE Healthcare). The fractions containing the respective dDia1 construct were pooled and dialyzed against storage buffer (150 mM KCl, 1 mM DTT, 60% glycerol and 20 mM HEPES pH 7.4) and stored at −20 °C. Cloning and expression of dDia3 P4C reference construct (amino acids 1009–1561) will be described elsewhere. *D. discoideum* heterodimeric capping protein Cap32/34 (CP) and DdPFN isoforms I–III were purified as described previously (Schirenbeck et al., 2005; Breitsprecher et al., 2008). Actin was extracted from rabbit skeletal muscle according to Spudich and Watt (1971), and was labeled on Cys374 with ATTO488.

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