FEBS Letters 584 (2010) 1111–1118

FEBS Letters

journal homepage: www.FEBSLetters.org

Mapping of the basic amino-acid residues responsible for tubulation and cellular protrusion by the EFC/F-BAR domain of pacsin2/Syndapin II

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ARTICLE INFO

Article history:
Received 15 January 2010
Revised 10 February 2010
Accepted 18 February 2010
Available online 24 February 2010

Edited by Felix Wieland

Keywords:
Membrane
Protrusion
Invagination
EFC domain
F-BAR domain

ABSTRACT

The extended Fes-CIP4 homology (EFC)/FCH-BAR (F-BAR) domain tubulates membranes. Overexpression of the pacsin2 EFC/F-BAR domain resulted in tubular localization inside cells and deformed liposomes into tubules in vitro. We found that overexpression of the pacsin2 EFC/F-BAR domain induced cellular microspikes, with the pacsin2 EFC/F-BAR domain concentrated at the neck. The hydrophobic loops and the basic amino-acid residues on the concave surface of the pacsin2 EFC/F-BAR domain are essential for both the microspike formation and tubulation. Since the curvature of the neck of the microspike and that of the tubulation share similar geometry, the pacsin2 EFC/F-BAR domain is considered to facilitate both microspike formation and tubulation.

Structured summary:
MINT-7710892: *EFCS pacsin2* (uniprotkb:Q9UNF0) and *EFCS pacsin2* (uniprotkb:Q9UNF0) *bind* (MI:0407) by *X-ray crystallography* (MI:0114)

1. Introduction

BAR domain superfamily proteins deform membranes to a geometry corresponding to the structures of the membrane-binding surface of the BAR-superfamily, and/or bind to the membranes that fit their structures, and thus function to generate specific membrane geometries [1–4]. The BAR domain forms a crescent-shaped dimer, with a positively charged, concave surface [5]. The positively charged surface of the domain binds to the negatively charged inner surface of the plasma membrane to form invaginated tubular membrane structures. Furthermore, the BAR domains from endophilin and amphiphysin have hydrophobic amino-acid residues that are inserted into the membrane on the concave surface or dimer ends [5–7]. The EFC (or F-BAR) domains from CIP4, Toca-1, and FBP17 form crescent-shaped dimers, and their concave surfaces bind to the membrane [8–10]. The F-BAR domain of FCHo2 also forms a crescent-shaped dimer, but the curvature of its membrane binding, concave surface is larger than those of the EFC/F-BAR domains of CIP4 and Toca-1 [11]. In contrast to the EFC/F-BAR domains of CIP4 and Toca-1, the lateral surface of the F-BAR domain of FCHo2 is curved [11]. The F-BAR domain of srGAP2 and the F-BAR-FX unit of Fes/Fer are involved in the formation of cellular protrusions [12,13], but the underlining mechanisms are unclear because the structures remain unsolved.

Pacsin/Syndapin forms one branch of the EFC/F-BAR domain protein family [2,8,14–16]. Pacsins/Syndapins function in the morphogenesis of neurons and in zebrafish notochord development, presumably through endocytosis and/or protrusive structure formation [17,18]. Recently, the structures of the EFC/F-BAR domains of human pacsin1/Syndapin I, pacsin2/Syndapin II, and Drosophila pacsin/Syndapin were reported [17,19], but the formation of cellular structures by these domains was not described. We also solved the structure of the pacsin2 EFC/F-BAR domain, at higher resolution. Here we describe the membrane interactions of the EFC/F-BAR domain of pacsin2/syndapin II for both tubulation and filopodia-like process formation in cells.

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0014-5793/$36.00
doi:10.1016/j.febslet.2010.02.058

2. Materials and methods

2.1. Liposome assays

The liposome-binding assay was performed as previously described [5,9,20]. Liposomes were prepared from total bovine brain lipids (Folch fraction 1, Avanti Polar Lipids) [21]. Dried lipids, under nitrogen gas, were resuspended in XB (10 mM Hepes [pH 7.9], 100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, and 5 mM EGTA) by mixing with a vortex, followed by hydration at 37 °C for 1 h. No sucrose was added to the XB. This preparation yielded a mixture of liposomes with various diameters (0.1–2 μm), and some of the liposomes were large multi-lamellar vesicles. Liposome co-sedimentation assays were performed as follows. To

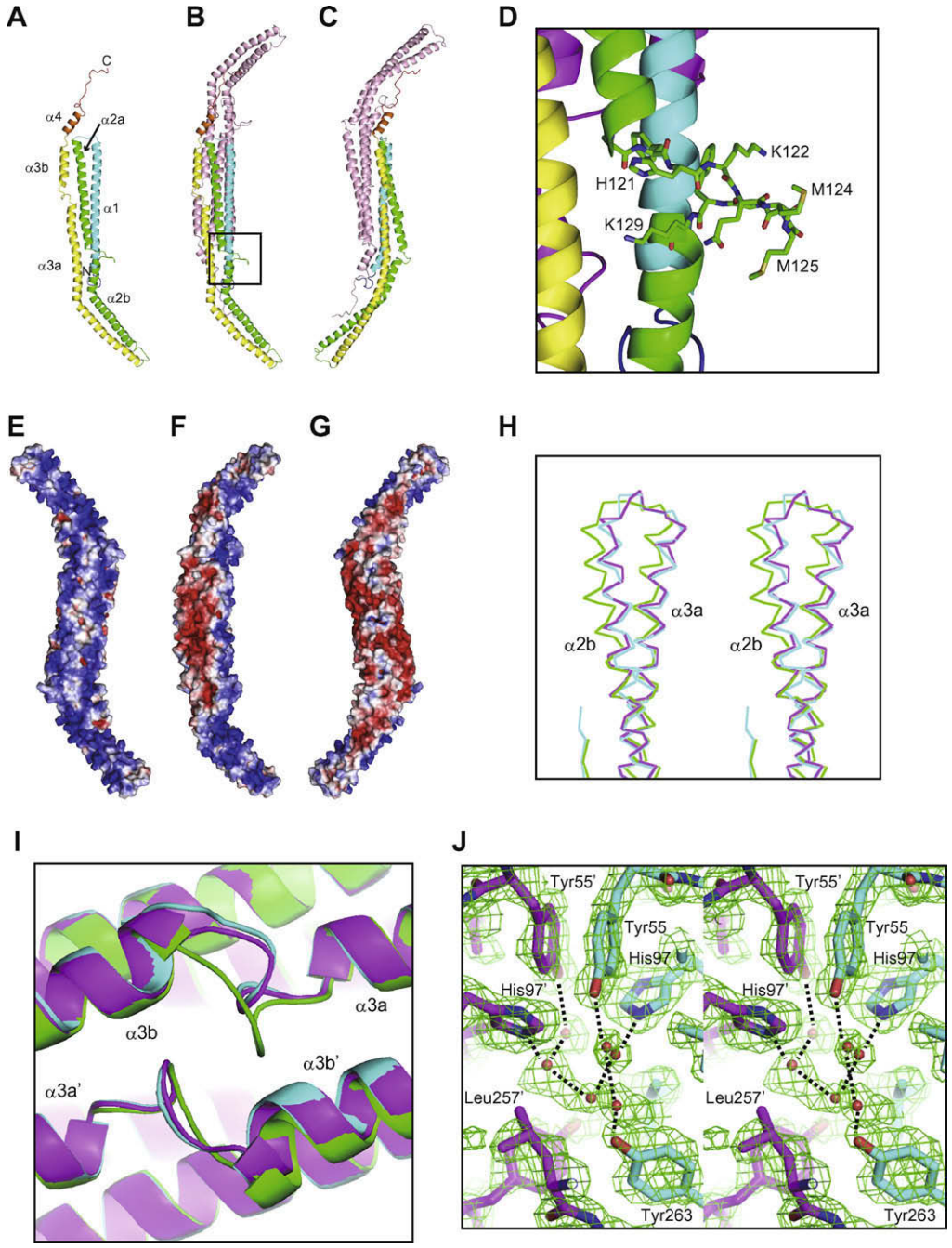

Fig. 1. Three-dimensional structure of the pacsin2 EFC/F-BAR domain. (A) Ribbon diagram of the structure of $EFCS_{pacsin2}$ (residues 1–305). N and C indicate the amino and carboxyl termini of the molecule, respectively. The secondary structure elements are colored differently. (B) Side view of the $EFCS_{pacsin2}$ dimer. One molecule is depicted as in (A), while the other molecule is colored magenta. The box indicates the region shown as a larger image in (D). (C) Top view of the $EFCS_{pacsin2}$ dimer. The dimer is rotated by 90° relative to (B). (D) Residues in the insertion loop between helices α2a and α2b are shown as stick representations. (E–G) Electrostatic potential surfaces of the $EFCS_{pacsin2}$ dimer, indicated with blue as positive and red as negative. (E) Bottom view (concave side); (F) side view; (G) top view (convex side). (H, I) Superpositions of $EFCS_{pacsin2}$ (cyan), $EFCL_{pacsin2}$ (magenta), and the previously determined pacsin2 EFC domain (green). Secondary structures are indicated. (H) The stereo view of the Cα traces around the tips of one coiled-coil region is shown. (I) The ribbon models around the loop region connecting helices α3a and α3b are shown. The prime symbol (′) denotes secondary structures in the dimer-related molecule. (J) A stereo view of a $2F_o–F_c$ omit electron density map, contoured at 1.5σ, of a representative dimer interface of $EFCS_{pacsin2}$. The first and second molecules in the dimer interface are shown as stick models and are colored cyan and magenta, respectively. The hydrogen bonding network in the dimer interface, involving several water molecules, is shown. Hydrogen bonds are shown as dashed lines. Residues involved in the dimer interface are labeled. The prime symbol (′) denotes residues in the second molecule of the dimer.