F-BAR domains: multifunctional regulators of membrane curvature

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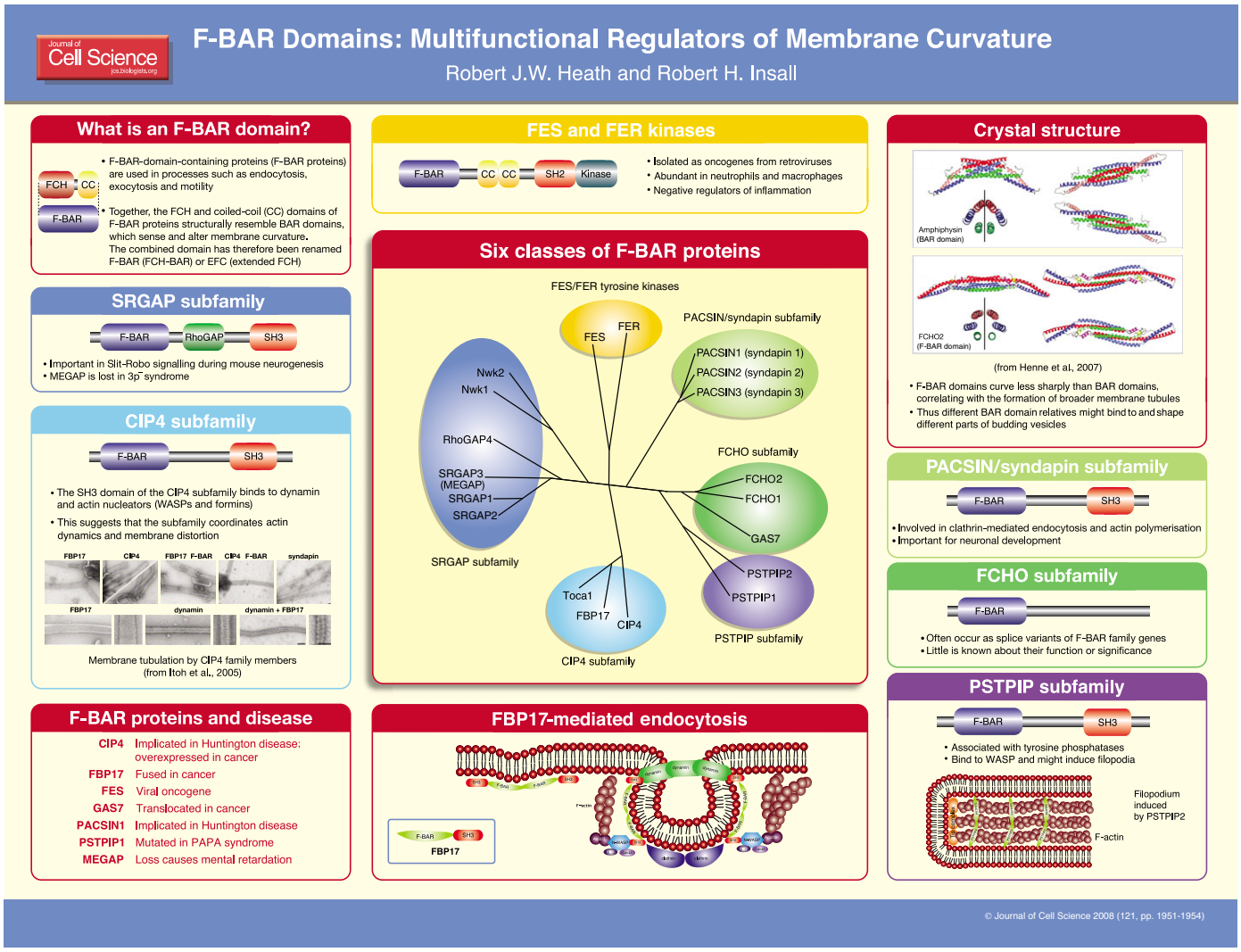
The F-BAR-domain-containing proteins (F-BAR proteins) are a group of adaptor proteins, members of which are found in all eukaryotes except plants. Also known as Pombe/Cdc15 homology (PCH)-family proteins, they have essential roles in fundamental biological processes, such as endocytosis, exocytosis and cell motility (Lippincott and Li, 2000; Greer, 2002). This Cell Science at a Glance article describes the emerging family of F-BAR proteins, and what is known about their structure, function and role in human disease.

The F-BAR domain and the family of F-BAR proteins

The FES-CIP4 homology (FCH) domain is the archetypal feature of all F-BAR proteins. This domain was originally identified while characterizing Cdc42-interacting protein 4 (CIP4; also known as TRIP10) (Aspenstrom, 1997). In F-BAR proteins a coiled-coil domain closely follows the FCH domain. The C-terminus can include various combinations of RhoGAP, kinase, SH2 and SH3 domains. Recent work has demonstrated that, together, the FCH and coiled-coil domains are structurally similar to Bin/amphiphysin/RVS (BAR) domains (Itoh et al., 2005; Tsujita et al., 2006). These are a family of α-helical membrane-binding modules that can detect, induce and be regulated by membrane curvature, and that function in endocytosis, regulation of the actin cytoskeleton and signalling (Itoh and De Camilli, 2006). On this basis these two domains have since been reclassified together as the F-BAR (FCH-BAR) or EFC (extended FCH) domain.

There are at least 21 F-BAR-protein-encoding genes in the human genome, which encode approximately 36 proteins. F-BAR proteins can generally be divided into six subfamilies: FES/FER tyrosine kinases, the PACSIN/syndapin subfamily, the CIP4 subfamily, the SRGAP subfamily, the PSTPIP subfamily and the FCH-domain-only (FCHO) subfamily (Aspenstrom et al., 2006; Chitu and Stanley, 2007). Primary sequence



(See poster insert)

homology between the family members is low (Lippincott and Li, 2000), and similarity is defined mainly by the predicted domain structure.

F-BAR proteins in endocytosis – insights from structural studies

A large body of evidence implicates F-BAR proteins – including formin-binding protein 17 (FBP17, also known as FNBP1), CIP4, transducer of Cdc42-dependent actin assembly 1 (Toca1; also known as FNBP1L), proline-serine-threonine phosphatase-interacting proteins 1 and 2 (PSTPIP1 and PSTPIP2, respectively) – in endocytosis (Kessels and Qualmann, 2004). The first link was the finding that the SH3 domain of some members of the F-BAR family (i.e. members of the PACSIN/syndapin and CIP4 subfamilies) can bind to dynamin (Itoh et al., 2005; Tsujita et al., 2006; Kessels and Qualmann, 2004), a GTPase that catalyses vesicle budding and scission of the lipid bilayer from intracellular membranes during clathrin-mediated endocytosis (Shafer and Voss, 2004). The most striking evidence for the involvement of F-BAR proteins in endocytosis came from the observation by Kamioka and colleagues (Kamioka et al., 2004), who reported that FBP17 induces tubular invaginations of the plasma membrane, and that the F-BAR domain is necessary and sufficient for this. A mutant form of FBP17 that is unable to bind to dynamin induces the formation of tubules that remain attached to the plasma membrane, leading the authors to conclude that the F-BAR domain acts in a manner similar to BAR domains and that an interaction with dynamin is required for endocytosis. This work was expanded by Itoh and colleagues and Tsujita and colleagues, who demonstrated that the F-BAR domain has an affinity for phospholipid-containing liposomes and induces membrane tubulation both in vivo and in vitro (Itoh et al., 2005; Tsujita et al., 2006). Thus, the F-BAR domain is likely to act as a membrane-targeting module during endocytosis.

Proteins of the N-terminal amphipathic helices BAR (N-BAR) family, a subset of the BAR-domain family, contain an N-terminal amphipathic helix that precedes the consensus BAR domain (Weissenhorn, 2005; Gallop et al., 2006). The hydrophobic face of the helix can insert into the hydrophobic phase of the membrane-lipid bilayer, displacing the phospholipids therein. This displacement induces membrane curvature, which is then stabilised by the BAR domain (Weissenhorn, 2005; Gallop et al., 2006; Masuda et al., 2006). The crystal structures of the N-BAR and BAR domains have revealed that they are crescent-shaped dimers binding to highly curved membranes (outer radius ~11-15 nm) via the concave surface of the protein (Gallop and McMahon, 2005). Basic residues on the concave surface bind to negatively charged lipids, such as phosphatidylserine or phosphatidylinositol (4,5)-bisphosphate, through electrostatic interactions (Gallop and McMahon, 2005). The recent determination of the crystal structures of the F-BAR domains of mammalian FCHO2, FBP17 and CIP4 reveals that these domains are also structurally similar to BAR domains – characteristically, an elongated dimer formed by the antiparallel interaction of two α-helical coiled coils, each a three-helical bundle (Henne et al., 2007; Shimada et al., 2007). This consolidates a trend among membrane-bending proteins that contain BAR, N-BAR or F-BAR domains: the consequence of domain dimerization is the formation of a central six-helical bundle, from which two helices protrude on either side. This structure generates a crescent-shaped molecule that has a family-specific radius of curvature.

Compared with those of the BAR domain, however, the helices of the F-BAR domain are relatively long and shallow [200-280 Å in diameter versus ~600 Å in diameter (Frost et al., 2007)]. This correlates with the difference in diameter of the tubules that are formed by BAR and F-BAR proteins (Itoh et al., 2005; Tsujita et al., 2006). In addition, BAR and F-BAR domains display preferences for differently sized liposomes in vitro, <100 nm for BAR domains and >100 nm for F-BAR domains (Henne et al., 2007). It is therefore possible to postulate that BAR, N-BAR and F-BAR proteins bind to and shape different parts of budding vesicles.

F-BAR proteins and the cytoskeleton

The most commonly reported function of F-BAR proteins in mammalian cells is cytoskeletal organisation, often in close proximity to the plasma membrane. The best-characterised interaction is between F-BAR proteins and Wiskott-Aldrich syndrome protein (WASP) or neuronal-WASP (N-WASP), which are regulators of the actin-nucleating Arp2/3 complex (Ho et al., 2004). This interaction has also been observed in the budding yeast *Saccharomyces cerevisiae*, in which the PSTPIP orthologue Bzz1p binds to the WASP orthologue Las17p (Soulard et al., 2002). In resting mammalian cells, members of the WASP-interacting protein (WIP) family sequester N-WASP in an auto-inhibited conformation that masks the C-terminal Arp2/3-binding site. Upon stimulation, activated Cdc42 interacts with the F-BAR protein Toca1 as well as with N-WASP and WIP, causing dissociation of the N-WASP–WIP complex and activation of N-WASP. The proteins of the PACSIN/syndapin subfamily have roles in both membrane trafficking and reorganization of the actin cytoskeleton (Kessels and Qualmann, 2004). Syndapin binds to dynamin, synaptojanin and N-WASP through its C-terminal SH3 domain (Modregger et al., 2000; Kessels and Qualmann, 2004; Peter et al., 2004). PSTPIP2 has been shown to bundle actin filaments, to induce the formation of filopodia and to inhibit ruffling in macrophages (Chitu et al., 2005).

In *Schizosaccharomyces pombe* the F-BAR protein Cdc15p recruits the formin Cdc12p and the Arp2/3 activator Myo1p to the central region of the cell during cytokinesis, orchestrating the formation of the contractile actomyosin ring (Carnahan and Gould, 2003). In *S. cerevisiae* the CIP4 orthologue Hof1p/Cyk2p has a similar role in the formation of the actomyosin ring at the bud site (Lippincott and Li, 1998). Hof1p/Cyk2p has several binding partners that are involved in cytoskeletal organisation, including the verprolin Vrp1p and the formin Bnr1p. Thus, regulation of the actin cytoskeleton by F-BAR proteins is conserved from yeast to higher eukaryotes.

F-BAR proteins and disease

Mammalian F-BAR proteins have been identified in additional biological processes, in which disturbed functionality is associated with disease. For instance, the SRGAP subfamily protein MEGAP (also known as WRP and SRGAP3) is functionally inactivated in patients who have deletion 3p ($3p^{-}$) syndrome, a severe mental retardation disorder (Endris et al., 2002). The SH3 domain of MEGAP is reported to bind to SCAR1/WAVE1 and to

have Rac-specific GTPase activity (Soderling et al., 2002). Why the loss of MEGAP causes 3p$^-$ syndrome is unknown, but MEGAP is expressed in foetal and adult brain tissue (Endris et al., 2002) and might regulate cell migration (Yang et al., 2006), suggesting that the phenotypes of 3p$^-$ syndrome are a result of impaired neuronal migration and axonal connectivity.

An additional role of F-BAR proteins has been identified in inflammatory disorders, in which there are several examples of mutations that result in disease. The most studied example is that of PSTPIP1 in pyogenic arthritis, pyoderma gangrenosum and acne (PAPA) syndrome (Wise et al., 2002), which is a rare autoinflammatory disease that causes destructive inflammation of skin, muscle and joints. Ulcerative lesions and cystic acne may also be present in afflicted individuals.

PSTPIP1 is regulated by phosphorylation, and two independent mutations in *PSTPIP1* (A230T and E250Q) that are found in patients with PAPA syndrome indirectly cause hyperphosphorylation of PSTPIP1. Why these mutations cause PAPA syndrome is unclear. PSTPIP1 forms a ternary complex with the protein tyrosine phosphatase PTP-PEST and CD2, a negative regulator of T-cell-receptor activity. This CD2–PSTPIP1–PTP-PEST complex counteracts the activation of the T-cell receptor. The presence of mutated PSTPIP1 abolishes this functional complex and thus causes increased activity of the α-chain of the T-cell receptor, resulting in increased interleukin 1 beta (IL1B) production by peripheral blood mononuclear cells (Shoham et al., 2003). It is possible that this misregulation is the basis of PAPA syndrome.

A second basis for PAPA syndrome might be the binding of the PSTPIP–PTP-PEST complex to Fas ligand (FasL). FasL is a member of the family of tumour-necrosis-factor-like ligands, induces apoptosis and has a role in the inflammatory response (Chitu and Stanley, 2007; Aspenstrom et al., 2006). The SH3 domain of PSTPIP1 can interact with a polyproline region of FasL, sequestering it in the secretory lysosomes of cytotoxic T cells and natural killer cells. Sequestration of FasL nullifies its function as a ligand, as it cannot be presented correctly at the cell surface (Qian et al., 2006). Another PSTPIP subfamily member, PSTPIP2, has also been implicated in macrophage autoinflammatory conditions in mouse models (Ferguson et al., 2006; Grosse et al., 2006).

There are early signs that F-BAR proteins are implicated in cancer and neurodegenerative disease. The genes encoding both FBP17 and GAS7 can form gene fusions with the MLL (mixed lineage leukaemia) gene (see Aspenstrom et al., 2006). CIP4 has been implicated in kidney cancer and renal cancer. CIP4 and PACSIN can bind the huntingtin protein, which is mutated in patients with Huntington disease, and both genes are upregulated in the brains of these patients. However, the mechanistic connections between F-BAR proteins and huntingtin are unclear.

Conclusion

The study of F-BAR proteins is in its infancy. F-BAR family members are being identified in an ever-increasing number of pathways. Several studies have begun to decipher the function of individual proteins, but many issues remain to be resolved. What are the molecular mechanisms that underlie the regulation of the actin cytoskeleton by F-BAR proteins? Do signalling cues activate these proteins, and if so, how? What is the precise function of the F-BAR domain and is that function conserved among the family members?

Overexpression studies have revealed that F-BAR domains are potent inducers of membrane curvature and have shown that expression of F-BAR proteins must be tightly regulated, because both over- and underexpression result in obvious phenotypes. However, there are few studies that address how these proteins are regulated in vivo. Perhaps knockdown and gene-disruption studies, in combination with structural and biochemical analyses, will help to decipher the complicated nature of this multifaceted protein family.

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