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Ever-Expanding Network of Dynamin-Interacting Proteins

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Abstract

Clathrin-mediated endocytosis is a major cellular pathway for internalization of proteins and lipids and for recycling of synaptic vesicles. The GTPase dynamin plays a key role in this process, and the proline-rich domain of dynamin participates in various protein–protein interactions to ensure a proper coordination of endocytic processes. Although dynamin is not directly associated with actin, several dynamin-binding proteins can interact with actin or with proteins that regulate actin assembly, thereby coordinately regulating actin assembly and trafficking events. This article summarizes dynamin interactions with various Src homology 3-containing proteins, many of which are actin-binding proteins. It also discusses the recently identified two new dynamin binding proteins, SH3 protein interacting with Nck, 90 kDa/Wiskott-Aldrich syndrome protein interacting with SH3 protein (SPIN90/WISH) and sorting nexin 9, and outlines their potential role as a link between endocytosis and actin dynamics.

Index Entries: Clathrin-mediated endocytosis; dynamin; proline-rich domain; src homology 3; SPIN90/WISH; sorting nexin 9; actin.

Dynamin As a Key Player in Clathrin-Mediated Endocytosis

Dynamin plays a critical role in clathrin-mediated endocytosis, a major cellular pathway for internalization of proteins and lipids and for recycling of synaptic vesicles *(1–5)*. Dynamin oligomerization at the necks of invaginated clathrin-coated pits stimulates GTPase activity, which leads to constriction and release the vesicle from the membrane *(6)*. Dynamin comprises several domains. The N-terminal nucleotide-binding domain is responsible for GTP hydrolysis, and C-terminal proline-rich domain (PRD) links dynamin to the Src homology 3 (SH3) domains of multiple endocytic proteins, including amphiphysin *(7)*,

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endophilin *(8)*, and syndapin *(9)*. The central pleckstrin homology (PH) domain controls dynamin's binding to membrane phospholipids *(10)*, and a coiled-coil domain (also called the GTPase effector domain) that follows the PH domain may play an important role in dynamin self-assembly and regulation of GTPase activity. The interaction force between the PH domain and lipid is not enough to recruit dynamin from the cytosol to plasma membrane *(11)*. Indeed, the truncation of PRD in dynamin was known to block the endocytosis by mislocation of protein *(10)*. Therefore, PRD–SH3 interaction between dynamin and other endocytic molecules is important for proper localization and function of dynamin.

Recent studies have suggested that dynamin may play multiple roles beyond its role as a mechanochemical enzyme in endocytosis *(12)*. One mediator is actin, and a connection of dynamin to actin function is of significant interest. The mechanism by which dynamin affects actin dynamics during endocytosis is unclear, but some clues have emerged. Recent studies place dynamin on actin comets generated either by *Listeria* or by type I phosphatidylinositol 5-phosphate kinase *(13)*. Evanescent field microscopic study showed that the inward movement of vesicle occurred immediately after a brief burst of dynamin recruitment and that it was accompanied by transient actin assembly *(14)*. Endocytic vesicles may use actin polymerization to move into the cytosol after being pinched off from the plasma membrane *(13,14)*. Several proteins containing SH3 that bind with the PRD of dynamin can also interact with actin directly. These include profilin *(15)*, actin-binding protein 1 (Abp1) *(16)*, and cortactin *(17)*. Profilin was the first identified protein that interacts with dynamin. Besides dynamin, profilin also binds phosphatidyl inositol 4,5-bisphosphate (PtdIns[4,5]P_2), Neural-Wiskott-Aldrich syndrome protein (N-WASP), the Arp2/3 complex *(15,18)*, and G-actin *(19)*. Abp1 and cortactin (both are F-actin-binding proteins) also bind the PRD of dynamin through their C-terminal SH3 domains *(16,20)*.

A second family of dynamin binding proteins interacts with actin indirectly. These include syndapin *(9)*, intersectin *(21)*, Grb2 *(22)* and Nck *(23)*. Intersectin contains multiple SH3 domains that interact with dynamin as well as N-WASP and synaptojanin. It also contains the Dbl homology domain, which functions as a guanine nucleotide (GDP) exchange factor to activate Cdc42, which in turn stimulates actin nucleation through N-WASP–Arp2/3 complex. Although syndapin contains one SH3 domain in its C-terminal region, it can dimerize itself, thus allowing it to interact with dynamin and N-WASP simultaneously *(24)*. Both N- and C-terminal SH3 domains of Grb2 can interact with dynamin as well as N-WASP, whereas Nck interacts with dynamin through its third SH3 domain, and with N-WASP and WASP-interacting protein (WIP) through its second SH3 domain *(22,25–27)*.

Because all these proteins contain the SH3 domain that interacts with dynamin's PRD domain, these results have suggested that the PRD of dynamin is a critical determinant for the interaction of this protein with the actin cytoskeleton and thus links endocytosis to the actin polymerization.

SH3 Protein Interacting With Nck, 90 kDa/Wiskott-Aldrich Syndrome Protein Interacting With SH3 Protein

SPIN90/WISH is a recently cloned protein, originally identified as a binding partner of Nck and Ash/Grb2, respectively *(28,29)*. It contains an N-terminal SH3 domain, three central PRDs, and a hydrophobic C-terminus, and each of these domains is a potential site of protein–protein interaction *(28,30)*. SPIN90/WISH is known to participate in sarcomere assembly during cardiac myocyte differentiation, to function as an important regulator of actin dynamics *(28)*, and, in concert with Nck, to form a novel complex with β-p21 activating kinase exchange factor (β-PIX) and WASP for

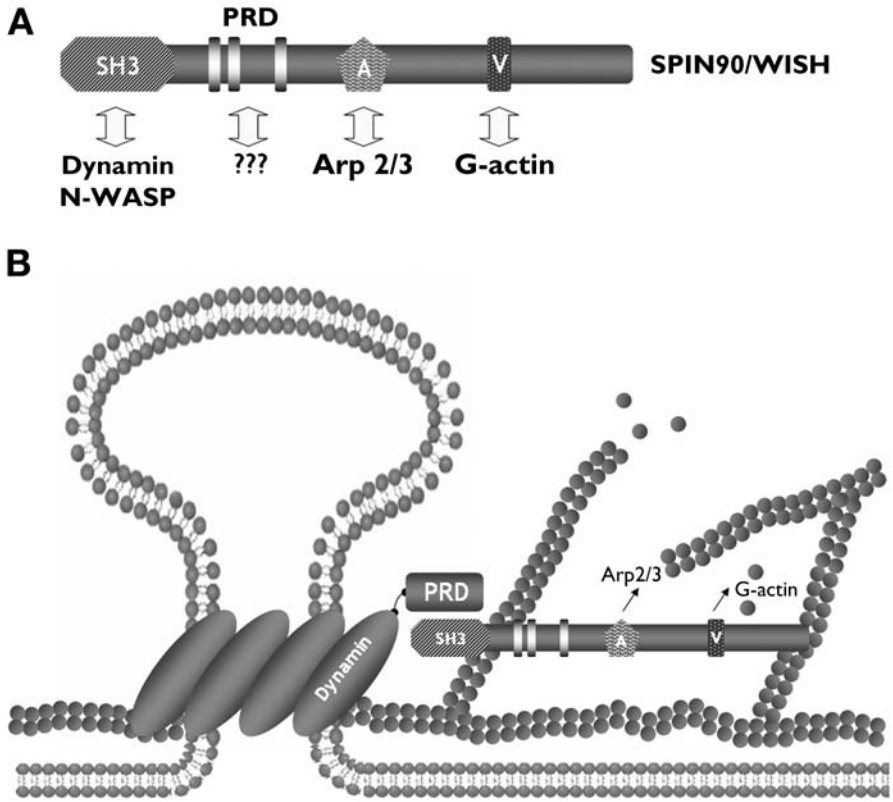

Fig. 1. The domain structure of SPIN90/WISH and its potential role in clathrin-mediated endocytosis. (A) Schematic diagram of SPIN90/WISH. The protein that interacts with each domain is indicated below the arrow. A, acidic-like domain; V, VPH-like domain. (B) Model showing interaction between dynamin and SPIN90/WISH as it might occur at the plasma membrane during clathrin-mediated endocytosis. Dynamin wrap around the membrane, and its extended PRD can bind the SH3 domain of SPIN90/WISH. In turn, SPIN90/WISH regulates actin polymerization and organization via its C-terminal acidic-like domain and VPH-like domain, linking the endocytosis and actin cytoskeleton.

stable cell adhesion at focal contacts triggered by integrin β_{1A} *(30)*.

SPIN90/WISH binds N-WASP through its N-terminal SH3 domain and is able to strongly enhance N-WASP-induced Arp2/3 complex activation, resulting in rapid actin polymerization that is required for microspike formation *(29,31)*. Interestingly, SPIN90/WISH can activate actin polymerization—even in N-WASP-depleted extracts—suggesting that SPIN90/WISH can activate Arp2/3 complex through both N-WASP-dependent and -independent pathways without Cdc42 *(29)*. It is not known, however, how it regulates actin polymerization independently of N-WASP. Recently, our studies indicated the SPIN90 C-terminus contains an A-like domain and a verprolin homology (VPH) domain, which are functionally equivalent to the VCA domain of WASP family proteins, inducing activation of the Arp2/3 complex. Indeed, the immunohistochemical and in vitro binding assays showed that it associates with the Arp2/3 complex via its C-terminal A-like domain and binds directly to G-actin through its VPH-like domain, bringing about actin polymerization in vitro *(31)*. SPIN90/WISH polymerizes actin in vitro and induces actin

comet formation in COS-7 as well as in BHK cells *(31)*. Therefore, these results strongly suggest that the SPIN90/WISH C-terminal hydrophobic region is responsible for inducing actin polymerization in the N-WASP-independent pathway *(31)*.

Therefore, SPIN90/WISH may play a key role not only in signal pathway from cell adhesion but also in the re-organization of the actin cytoskeleton. However, few in vivo ligands for the SH3 domain PRD of SPIN90/WISH have been identified. Notably, SPIN90/WISH has a reverse domain organization similar to that of cortactin or Abp1, with SH3 domain and PRD in its N-terminus and actin- and Arp2/3-binding regions (VPH and A-like domains) in its C-terminus (Fig. 1A).

We recently identified dynamin I as a binding partner of SPIN90/WISH in neurons. The kinetics of synaptic vesicle endocytosis could be modulated by their interactions. Overexpression of SPIN90/WISH or its SH3 domain affects the synaptic vesicle endocytosis in neurons. The knockdown of endogenous SPIN90/WISH also significantly inhibits the synaptic vesicle endocytosis *(32)*. The specificity of interaction between the PRD of dynamin I and SH3 domain of SPIN90/WISH was confirmed by a series of biochemical and physiological experiments. Interestingly, SPIN90/WISH affects endocytosis only after stimulation, without affecting the rate of endocytosis during the stimulation. Therefore, SPIN90/WISH appears to play a role in the conventional clathrin-mediated endocytosis rather than in a "kiss-and-run"-type rapid retrieval of synaptic vesicle. SPIN90/WISH expression also inhibits the uptake of transferrin in CHO cells (SC, unpublished data). Our results strongly suggest that SPIN90/WISH binds dynamin via its SH3 domain, thus participating in clathrin-mediated endocytosis, whereas via its C-terminal hydrophobic regions, it associates with Arp2/3 complex and G-actin, thus regulating actin polymerization. It also raises the possibility that SPIN90/WISH may play a role as a link between the actin dynamics and the clathrin-mediated endocytois.

Sorting Nexin 9

Sorting nexin 9 (SNX9), which is also known as SH3PX1, is a member of sorting nexin superfamily distinguished by the presence of a phosphoinositide-specific Phox domain. The sorting nexin family protein contributes to protein sorting in cells by its ability to bind specific lipid and to form protein–protein complexes. SNX9 was initially identified as an interactive protein of the metalloprotease MDC9 and MDC15 *(33)*. In *Drosophila,* it functions in connection of cytoskeletal structure to the receptor-like molecule Dscam trafficking *(34)*. SNX9 is composed of N-terminal SH3 domain, which binds several PRD-containing proteins in cells, low-complexity regions, Phox domain (which binds phospholipids), and C-terminal Bin/Amphiphysin/Rvs (BAR) domain (which is involved in lipid binding, curvature sensing, and protein dimerization) *(35–37)* (Fig. 2A).

Recent studies have shown that SNX9 forms a complex with dynamin in cytosol and regulates the recruitment of dynamin to the membrane *(38)*. SNX9 forms in an inactive ternary complex with dynamin 2 and the metabolic enzyme aldolase in the cytosol. Upon phosphorylation of SNX9, the SNX9-dynamin 2 assembly is released from aldolase and can now bind to the membrane, as shown by a biochemical assay *(36)*. Overexpression of dominant negative constructs of SNX9 affects the uptake of transferrin *(35)*. The knockdown of SNX9 expression by siRNA significantly inhibits the membrane recruitment of dynamin *(36)* and decreases the uptake of transferrin *(39)*. Furthermore, SNX9 enhances dynamin's assembly and increases dynamin's GTPase activity *(39)*. Other endocytic molecules known to play key roles in clathrin-mediated endocytosis, adaptor protein complex 2 and clathrin, also bind to the low-complexity region of SNX9 in a cooperative manner *(35)*. These

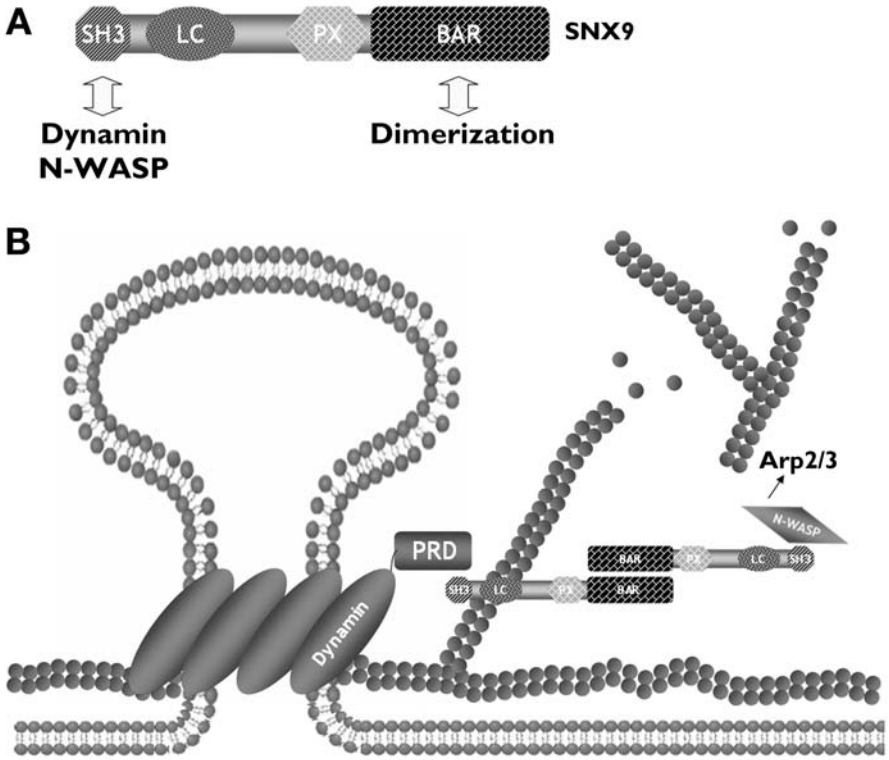

Fig. 2. The domain structure of SNX9 and its potential role in clathrin-mediated endocytosis. **(A)** Schematic diagram of SNX9. The protein that interacts with each domain is indicated below the arrow. **(B)** Model showing interaction between dynamin and SNX9 as it might occur at the plasma membrane during clathrin-mediated endocytosis. Dynamin wrap around the membrane and its extended PRD can bind the SH3 domain of SNX9. SNX9 dimerizes with each other via its BAR domain and interacts with N-WASP via its SH3 domain, regulating actin polymerization through Arp 2/3 complex. (*See* text for detailed description.)

results show that SNX9 is required for efficient clathrin-mediated endocytosis, and the biochemical characterization supports a model for the mechanistic function of SNX9. Our recent data indicate that SNX9 associates with dynamin-1 in neurons and plays a regulatory role in synaptic vesicle endocytosis (SC, unpublished data).

SNX9 contains a putative BAR domain in its C-terminal region. The BAR domain-containing proteins bind to lipids and to bend membranes, which is a critical step for clathrin-mediated endocytosis. Indeed, proteins that are involved in endocytosis (such as dynamin, amphiphysin, and endophilin) have been shown to tubulate membranes on their own in vitro in a BAR-domain-dependent manner (a detailed review can be found in ref. *3*). The expression of SNX9 shows a strong membrane tubulating activity in COS-7 cells (SC, unpublished data). SNX9 also can dimerize itself via the BAR domain.

Besides dynamin, the SH3 domain of SNX9 binds the PRD of WASP or N-WASP (ref. *34*; SC, unpublished data) so that it can regulate actin polymerization. Because SNX9 can be dimerized and binds dynamin at one end and N-WASP at the other end, these results raise the possibility that SNX9 plays a role as a link between the actin dynamics and clathrin-mediated endocytosis (Fig. 2B). However, further work is needed to determine whether SNX9 might also play a role

in regulating actin assembly, in addition to its role in early endocytosis with dynamin.

Conclusion

Fifteen years after its discovery, the function of dynamin in clathrin-mediated endocytosis has been extensively studied, but much remains unknown. Whether it is a mechanochemical enzyme that pinches the vesicle from the membrane or just a regulator to recruit effectors of endocytosis, it is difficult to envision that a single PRD of dynamin is involved in the interactions with more than 10 different proteins, and each interaction is important to regulate endocytosis. As the list of dynamin's new binding partners continues to expand, the major challenges for the future are to determine the roles played by dynamin with various binding partners at different steps in clathrin-mediated endocytosis and to determine how these interactions and the actin cytoskeleton are coordinated and regulated.

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