HHS Public Access

Author manuscript

Trends Cell Biol. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

Trends Cell Biol. 2017 March ; 27(3): 189–200. doi:10.1016/j.tcb.2016.11.001.

Endocytosis, metastasis and beyond: Multiple facets of SNX9

Nawal Bendris[1,*] and **Sandra L. Schmid**[1,*]

[1]Department of Cell Biology, The University of Texas Southwestern Medical Center, Dallas. 6000 Harry Hines Blvd. Dallas, TX 75390-9039

Abstract

Sorting nexin 9 (SNX9) was first discovered as an endocytic accessory protein involved in clathrin-mediated endocytosis. However, recent data suggest that SNX9 is a multifunctional scaffold that coordinates membrane trafficking and remodeling with changes in actin dynamics to affect diverse cellular processes. Here we review the accumulated knowledge on SNX9 with an emphasis on its recently identified roles in clathrin-independent endocytic pathways, cell invasion and cell division, which have implications for SNX9 function in human disease, including cancer.

Keywords

actin; RhoGTPase; cell motility; invadosome; mitosis; inflammation; cancer

Protein scaffolds integrate cellular functions

Scaffold proteins typically encode multiple protein interaction domains and function to spatially and temporally coordinate divergent cellular activities. Scaffolds can increase the efficiency of signaling cascades by linking kinases with their substrates or regulatory GTPases with their effectors, and/or target these signaling events to specific locations within the cell. Other scaffold proteins gather and coordinate the functions of multiple factors required for protein sorting and membrane deformation to create sites for vesicle formation or delivery. Among the latter is sorting nexin 9 (SNX9, also named SH3PX1), first identified and best studied for its role in clathrin-mediated endocytosis. As we describe in this review, the SNX9 scaffold binds to specific cell surface receptors, recruits components of both the endocytic and actin regulatory machineries, and interfaces with signaling GTPases and kinases. Several recent studies have expanded the role of SNX9 suggesting that it functions to coordinate membrane trafficking and actin dynamics in diverse cellular processes such as migration, invasion, and cell division. Reflecting its role as the nexus of these processes, recent studies suggest that SNX9 can play a critical role in controlling cancer cell invasion and metastasis.

Corresponding authors: nawal.bendris@utsouthwestern.edu, sandra.schmid@utsouthwestern.edu, phone: 214-648-3941, Fax: 214-648-7491.

*No conflicts of interest are reported

Domain structure and biochemical properties of SNX9

SNX9 is a multidomain scaffold

SNX9 is a member of the sorting nexin (SNX) family of proteins that were first identified in the context of endosomal sorting [1,2]. The SNX9 subfamily includes SNX18 and SNX33 (also called SNX30). Sorting nexin family members share a common phox-homology (PX) domain that facilitates membrane binding *via* phosphatidylinositol (PtdIns) lipids, targeting them to different cellular compartments, most commonly the endosome through binding to PtdIns3P [3,4]. In addition to the PX domain, SNX9 subfamily members contain an SH3 domain at the N terminus, a low complexity (LC) domain, and a Bin–Amphiphysin–Rvs (BAR) domain at the C terminus (Figure 1A). The PX and BAR domains form a functional unit termed the PX-BAR module that mediates membrane interactions [5,6,7], including membrane deformation [3,4,8]. The BAR domain also mediates homo-dimerization between SNX9 family members [9]. Despite one report suggesting that these proteins can form heterodimers [10], other data suggest that this is unlikely at physiological levels of expression [11,12]. In addition to binding lipids, the PX domain can also mediate protein-protein interactions [13,14]. Given the multiple binding platforms provided by the SH3, LC, PX and BAR domains, SNX9 can be functionally classified as a protein- and lipid- binding scaffold, interacting with proteins involved in a variety of cellular processes (Table 1).

Membrane binding and curvature generation

While other PX and BAR domains show high specificity for binding to specific PtdIns lipids, the lipid-binding pocket of SNX9 is rather large explaining its broad range of interactions [14,15,16]. SNX9 binds equally well to liposomes bearing PtdIns(3,4,5)P_3, PtdIns(4,5)P_2, PtdIns(3,5)P_2, and PtdIns(3,4)P_2 [16], enabling it to be targeted to diverse cellular compartments. SNX9 or its PX-BAR module alone can elicit membrane tubulation when incubated with liposomes *in vitro* [5] and can lead to dramatic tubulation of the plasma membrane (PM) when highly overexpressed in cells [8,11,14,17]. Mutational analyses confirm the role of the PX and BAR domains in membrane binding and tubulation *in vitro* and in cells [16,18,19,20]. Upon membrane binding SNX9 forms higher-order structures in a BAR-domain dependent manner [16]. Given its comparatively weak ability to generate membrane curvature on its own [21], it is likely that SNX9 normally functions in cells as a curvature sensor to recruit other proteins through SH3 and/or LC domain interactions and coordinate their membrane remodeling activities [14].

Regulation of actin assembly

SNX9 binds to N-WASP through its SH3 domain thereby stimulating N-WASP to trigger Arp2/3-dependent polymerization of branched actin filaments [9,22]. SH3 domain interactions are necessary, but not sufficient for this activity as dimerization through the BAR domain and higher order assembly onto membranes enhances SNX9- and N-WASP-dependent actin polymerization [9,16]. Hence, SNX9 dimerization and lipid binding synergize to fully activate N-WASP. SNX9-dependent activation of N-WASP on liposomes depends on both their lipid composition [16] and their curvature [23]. In this way, SNX9 can activate actin assembly in the context of cellular processes involving membrane remodeling.

In addition to direct activation of N-WASP and actin nucleation, SNX9 can also affect cytoskeletal changes by indirectly regulating RhoGTPases. RhoGTPases, which are regulators of actin dynamics, are activated and inactivated by Guanine Nucleotide Exchange Factors (GEFs) and GTPase-Activating Proteins (GAPs), respectively. Increased SNX9 expression in cancer cells resulted in decreased activation of RhoA and increased activation of Cdc42 [20]. Reduced expression had the opposite effects; thus, SNX9 is a negative regulator of RhoA and a positive regulator of Cdc42. While SNX9 binds directly to both RhoA or Cdc42, the domain involved has not been identified and it does not directly regulate their GTPase activities. Rather, SNX9 indirectly prolongs the GTP-bound state of Cdc42 by inhibiting its GAP-mediated inactivation [20]. How SNX9 acts on RhoA remains unclear. One possibility is that SNX9 might influence the GTPase's subcellular distribution to locally enrich or exclude them from membrane subdomains where they encounter their GEFs and GAPs.

Activation of RhoGTPases leads to modifications in the actin cytoskeleton organization that often translate into changes in cell morphology. In insect cells, SNX9 depletion impaired lamellipodia formation, whereas its overexpression induced the formation of long tubules and membrane extensions [24]. Similarly, changes in expression levels of SNX9 in mammalian cells influence cell shape. Thus, in accordance with increased RhoA activation [20], breast cancer cells form blebs when SNX9 is depleted (Figure 2). Following SNX9 overexpression, cells form filopodia (Figure 2), corresponding to increased Cdc42 activation [20]. Thus, the SNX9 scaffold can coordinate the regulation of actin assembly with membrane remodeling events.

SNX9 and endocytic membrane trafficking

Clathrin-mediated endocytosis

The role of SNX9 in clathrin-mediated endocytosis (CME) has been extensively studied since its discovery [25,26]. SNX9's function in CME can be attributed to its curvature sensing and generation activities and to its protein-protein interactions [8,27]. In addition to binding dynamin through its SH3 domain, SNX9 binds to the coat proteins clathrin and adaptor protein 2 (AP2) through motifs in the LC domain. These interactions facilitate its specific recruitment to sites of CME [15,28].

Multiple lines of evidence suggest that SNX9's primary endocytic function is in the recruitment and/or activation of dynamin, which catalyzes membrane fission at late stages of CME. *In vitro* assays using liposomes as template showed that SNX9 potentiates the assembly-stimulated GTPase activity of dynamin [26] and helps to retain dynamin on liposomes during cycles of GTP hydrolysis and disassembly [29]. Although dynamin is also recruited early to nascent clathrin-coated pits (CCP) [30], SNX9 and dynamin appear together as a burst at late stages of CME, corresponding to vesicle scission [26,31] (Figure 1B). However, siRNA knockdown of SNX9 in various cell types only mildly affects either dynamin recruitment [15,26] or transferrin receptor CME [9,18,20,26,32,33]. This could reflect functional redundancy with other SNX9 family members or other SH3-BAR domain containing endocytic accessory proteins. Indeed, significant impairment of endocytosis is achieved when SNX9 and SNX18 are both depleted [18].

Other studies suggest a more nuanced role for SNX9 in CME. For example, SNX9 has an inhibitory effect on dynamin-dependent vesicle release in an *in vitro* assay for membrane fission [21]. SNX9 knockdown decreased the efficiency of CCP maturation [34] and alters the spatial clustering of CCPs, reducing the rate of re-initiation at endocytic 'hot-spots' [35]. Consistent with this, high temporal resolution studies on its recruitment to CCPs revealed that SNX9 was still detected at the PM even after vesicle scission [31], positioning it to potentially initiate assembly of a second CCP. Altogether, these investigations suggest that SNX9 has subtle functions at several stages during CME that are not fully understood.

Clathrin-independent endocytosis

SNX9 functions in several clathrin-independent endocytosis (CIE) pathways that are driven by actin polymerization. These pathways require N-WASP and many are activated downstream of Rho family GTPases. Cargo molecules taken up by CIE include CD44, the transmembrane receptor for hyaluronic acid [36,37], glycosylphosphatidylinositol-anchored proteins (GPI-APs), and some bacterial toxins [27,38]. Many of these pathways function independently of dynamin and some require GRAF1, a BAR domain-containing protein that also functions as a GAP for RhoA and Cdc42 [27,39]. mCherry-SNX9 dynamically localizes with GPI-GFP at the PM [9] (Figure 1B), and in accordance with the role of SNX9 as an indirect regulator of RhoA, siRNA-mediated knockdown of SNX9 decreases CD44 internalization. Moreover, SNX9 can compensate for GRAF1 and restore CIE [20].

SNX9 was initially identified as a direct binding partner of the pro-forms of ADAM9 and 15 [40], which are catalytically inactive precursors of these ADAM proteins involved in cell-cell and cell-matrix adhesion [41]. Given that the inactive forms of ADAM9/15 are mainly localized in the Golgi, if or how SNX9 influences their trafficking from the Golgi has yet to be studied. However, a subpopulation of endogenous SNX9 colocalizes with the Golgi apparatus [26] (Figure 1B). Interestingly, SNX9 was recently shown to be required for endocytosis of MT1-MMP, a cell surface matrix metalloprotease in human cancer cells, presumably by CIE as TfnR uptake was only mildly affected under the same conditions [20]. Indeed, MT1-MMP endocytosis requires dynamin and endophilin A2 [42], hallmarks of a CIE pathway [43].

SNX9 and macropinocytosis

SNX9 also colocalizes with plasma membrane-associated actin structures such as lamellipodia and membrane ruffles [9] (Figure 1B). Several growth factors induce membrane ruffling and their progressive enclosure into circular dorsal ruffles, which mediate macropinocytosis [44]. After PDGF stimulation, SNX9 partially colocalizes with actin at newly formed dorsal rings and then becomes concentrated in the center of these structures subsequent to ring constriction [9] and the formation of large macropinocytic vesicles. SNX9 depletion decreases the number of dorsal rings induced by PDGF leading to decreased actin-mediated bulk endocytosis [9]. Correspondingly, its overexpression increases the number of macropinosomes [45].

Macropinocytosis constitutes the main entry pathway for bacteria in nonphagocytic cells. During bacterial infection or invasion of epithelial cells, several bacterial proteins are

injected into host cells to facilitate pathogen entry by altering cellular processes such as membrane trafficking, actin cytoskeleton dynamics, and loosening of cell junctions. In this regard, EspF, which is injected into cells by Enteropathogenic Escherichia coli and SopB, which is injected by Salmonella, recruit SNX9 to the PM and induce SNX9 and actin-mediated membrane remodeling to enable bacterial entry [46,47]. Consistent with this, SNX9 depletion reduces bacterial entry into host cells [48,49].

Thus, in addition to its well-studied role in clathrin-mediated endocytosis, SNX9 functions to coordinate actin assembly with membrane remodeling to drive multiple alternate routes of endocytosis.

SNX9 function in cell migration and invasion

The above discussion places SNX9 at the crossroads of endocytosis, signaling and actin cytoskeleton organization, all of which can influence cell motility [50]. Indeed, SNX9 enhances the migration of cancer cells through collagen matrices *via* inhibition of the RhoA-ROCK pathway and activation of N-WASP directly and/or indirectly *via* activation of Cdc42 [20]. Correspondingly, in a chick embryo model of metastasis, SNX9 overexpression in human breast cancer cells increased their metastatic activity, whereas its depletion decreased metastasis [20]. Unexpectedly, the effects of SNX9 on cell migration through collagen matrices appear to be independent of membrane binding, as mutations in the PX domain that perturb SNX9-liposome interactions are fully active [20].

Highly invasive cancer cells express invadopodia, actin-rich membrane protrusions that cluster MT1-MMP for localized matrix degradation. Invadopodia enable cancer cells to penetrate the extracellular matrix surrounding tumors. Specific adaptor proteins such as TKS5 are recruited to invadopodia [51] and are necessary for their formation. SNX9 binds TKS5 most likely *via* its LC domain, is localized to invadopodia (Figure 1B) and negatively regulates both their formation and function [52] (. Thus, SNX9 depletion increased invadopodia number and their matrix-degrading activity, in part by decreasing internalization of MT1-MMPs and increasing their surface expression at invadopodia [52].

That SNX9 negatively regulates invadopodia formation, but is required for migration through collagen and for efficient metastasis in a chick embryo model [20], suggests a bimodal function for SNX9 in cancer cell metastasis. Indeed, these two activities of SNX9 are independently regulated through phosphorylation by the tyrosine kinase, Src. Five Src-dependent phosphorylation sites were identified in SNX9 with Y239 being the major phosphorylated residue (Figure 3) [52,53]. The biological consequences of the single phosphorylation sites have not been evaluated, although it was suggested that phosphorylation of Y287 within the PX domain might interfere with SNX9's membrane binding abilities [53]. Other studies have suggested that tyrosine phosphorylation within the SH3 domain of SNX9 decreases its binding affinity to N-WASP [22]. Functionally, the non-phosphorylatable mutant (in which all identified Src tyrosine phosphorylation sites were mutated to phenylalanine) was as efficient as WT-SNX9 in rescuing MT1-MMP internalization and matrix degradation following the depletion of endogenous SNX9,

whereas the same non-phosphorylatable mutant had dominant negative effects on cell migration through collagen [52].

Together these data suggest independent and potentially sequential roles for SNX9 in cancer progression and metastasis: an early, invadopodia- and MT1-MMP-dependent role in local invasion from the primary tumor, and a role in metastatic cell dissemination through the surrounding ECM (Figure 4). Consistent with this, more aggressive, late-stage primary lung cancer tumors express less SNX9 protein than their early stage counterparts [52]; whereas SNX9 expression levels were higher in breast cancer metastases than in their primary tumors [20]. It is likely that the scaffolding activity of SNX9 and its diverse sets of interactions function to spatially and temporally coordinate endocytic membrane trafficking, signaling and actin dynamics to differentially affect cell motility and invasion. As illustrated in Figure 3, these interactions in turn can be regulated by site-specific phosphorylation of SNX9 by FAK, ACK, Src and other kinases, to modulate its activities in response to extracellular stimuli.

SNX9 and cell division

Recent studies have implicated SNX9 in the regulation of cell division. siRNA-mediated knockdown of SNX9 inhibits progression through and completion of mitosis, leading to the generation of multinucleated cells [32,54]. The data suggest that SNX9 plays multiple roles in cell division that are both dependent and independent of endocytosis. For example, during cytokinesis in synchronized HeLa or U2OS cells SNX9 depletion reduced endocytosis of transferrin receptor as well as trafficking from the Golgi, both of which are required for the massive membrane remodeling required for cell division. SNX9 is also localized to the mid-body suggesting a role in abscission. SNX9 depletion also effects assembly of the contractile ring and recruitment of myosin-II, presumably due to defects in N-WASP-dependent actin assembly. In contrast, SNX9 depletion did not inhibit the residual CME that occurs during mitosis; however, it resulted in a delay in both chromosome alignment and segregation [32]. Interestingly, the clathrin heavy chain is localized to the mitotic spindle where it plays a key role in the mitotic spindle organization that is independent of its role in CME [55]. SNX9 is required for the recruitment of clathrin to the mitotic spindle [54] (Figure 1B) through interactions with its LC domain. A SNX9 mutant unable to bind clathrin had a dominant-negative effect on the organization of the mitotic spindle, without perturbing CME [54], again reflecting the independence and functional diversity of SNX9's scaffolding functions.

Redundant and distinct functions for SNX9 family members

Although SNX9 is by far the best characterized member of its subfamily, recent studies have provided evidence for both overlapping and distinct functions for its cousins, SNX18 and SNX33. Drosophila and C. elegans express only a single SNX9 family member (DSH3PX1 and Lst4, respectively), which function in phagosome maturation and clearance of apoptotic cells [56,57], autophagosome biogenesis [56] and axonal guidance, the latter through a mechanism that requires its interactions with N-WASP and Dscam [22,58]. Not all mammalian SNX9 family members share these functions, suggesting their functional divergence upon gene duplication and evolution. Indeed, despite a conserved domain

structure, sequence homology between the three mammalian SNX9 family members is rather weak (<40%) [11]. Nonetheless, all three isoforms bind membranes, dynamin and N-WASP. Thus, it is not surprising that they exhibit both partially overlapping and divergent functions. For example SNX18 and SNX9 have at least partially redundant functions in CME [12], such that depletion of both is required to strongly inhibit CME in Cos7 cells [18], but not in all cell types [32]. Similarly, overexpression of either family member results in increased macropinocytosis [45], albeit to differential extents. In contrast, only SNX18 has been shown to promote autophagosome formation in mammalian cells [59,60] and to localize to a subset of AP1 and PACS1-positive endosomes [11]. SNX9 and SNX18 have opposite roles in the regulation of matrix degradation: SNX9 knockdown increases, whereas SNX18 knockdown decreases matrix degradation at invadopodia [52].

The proteins also have differential and non-redundant functions in cell division. Individual depletion of any of the three SNX9 family members results in the formation of multinucleated cells [32], although cell division is perturbed at different stages [10,32]. Thus, while contractile ring formation is perturbed by knockdown of either protein, which likely reflects common, but not fully redundant, functions in actin assembly, only SNX9 and SNX33 are localized to the midbody and affect abscission. SNX9 is the only family member localized to the mitotic spindle. Consistent with their differential functions, the proteins are differentially localized in both interphase [11] and mitotic cells [32]. More studies are needed to better define the shared and divergent functions of SNX18 and SNX33.

SNX9 and Disease

Collectively, emerging studies identify SNX9 as a multifunctional scaffold coordinating membrane and actin dynamics to affect multiple cellular processes. Not surprisingly, SNX9 is linked to many human diseases, including Lowe syndrome [61], chronic inflammation [62,63], and cancer [20,52]. Interestingly, these links relate mostly to alterations in its expression levels rather than point mutations that impair its function. As a scaffold, changes in expression levels could result in internal competition for binding partners that might disrupt its ability to effectively coordinate their activities. Mouse models are needed to better define and catalogue the phenotypes associated with SNX9 loss and/or overexpression in both development and disease. Similarly, it will be important to study the role of phosphorylation and dephosphorylation in regulating SNX9 activity, as well as the kinases and phosphatases involved. In particular, whether the effects of increased expression or activity of Src, FAK and ACK kinases in cancer are, in part, related to alterations in SNX9 activity has yet to be determined.

Whereas the function of SNX9 in cell invasion and invadopodia formation can be directly linked to cancer metastasis, whether other roles for SNX9, for example in the regulation of mitosis and endo-lysosomal trafficking, could contribute to cancer aggressiveness has not been studied. The fact that SNX9 knockdown induces a delay in both chromosome alignment and segregation, as well as cytokinesis defects and multinucleation [32] suggests that *in vivo*, SNX9 downregulation in cancer cells might contribute to the malignant phenotype by increasing genomic instability. In this regard, it was proposed that the clearance of apoptotic cells within tumors might also constitute a lateral transfer of genetic

material [64]. Given that SNX9 family members are required for apoptotic cell clearance, defects in this process may also contribute to cancer progression (for review see [57,65]). Alternatively, apoptotic cell clearance can trigger an immune response against tumor cells [66]. Although SNX33 can rescue apoptotic clearance in lst-4 deficient *C. elegans* [56], but whether SNX9 family member(s) participate in phagosome maturation and apoptotic cell clearance in human cells remains to be determined. Nonetheless, these diverse and potentially detrimental or beneficial roles for SNX9 in cancer progression may account for the variability of SNX9 expression levels in primary tumors and metastases reported in current databases (e.g. http://www.proteinatlas.org). More experimental work and *in vivo* data are needed to understand the underlying roles of SNX9 in disease.

Concluding Remarks

Complex cellular processes, including intracellular transport, cell migration, signaling, mitosis and cytokinesis, often require the spatially and temporally coordinated activity of diverse cellular machineries. Hence scaffold proteins that link these diverse components play essential roles in orchestrating these complex events. SNX9 has emerged as one such scaffold able to link membrane remodeling, signaling and actin dynamics in the context of diverse cellular processes. A complete SNX9 interactome, potentially obtained under different conditions or during distinct stages of the cell cycle would help to better define the multifunctional nature of its scaffolding activities. Whether SNX9 passively interacts with its partners or alters their activity either directly (as is the case for N-WASP) or indirectly (as appears to be the case for Rho-family GTPases) remains to be determined. Many questions also remain as to how SNX9 itself is regulated and activated for discrete events and which of its many interactions are required to affect which cellular processes (see Outstanding Questions). Mapping and mutating protein interaction and/or regulatory sites on SNX9, coupled with new methodology for genome modification both in cultured cells and whole animals will help to deconvolute SNX9's function in different cellular contexts. Animal models may also help to define SNX9s function in disease.

Acknowledgments

We thank members of the Schmid lab for helpful discussions. This work was supported by NIH grants GM42455 and MH61345 to S.L.S., N.B. was partially supported by a CPRIT training grant RP140110.

Abbreviation list

ADAM	A disintegrin and metalloprotease
ACK2	Activated Cdc42 Kinase 2
BAR domain	Bin–Amphiphysin–Rvs domain
CIE	Clathrin-independent endocytosis
CME	Clathrin-mediated endocytosis
LC domain	Low complexity domain

PtdIns	phosphatidylinositol
PX	Phox homology
SNX9	Sorting nexin 9

References

1. Kurten RC, Cadena DL, Gill GN. Enhanced degradation of EGF receptors by a sorting nexin, SNX1. Science. 1996; 272:1008–1010. [PubMed: 8638121]
2. Carlton J, Bujny M, Rutherford A, Cullen P. Sorting nexins--unifying trends and new perspectives. Traffic. 2005; 6:75–82. [PubMed: 15634208]
3. Cullen PJ. Endosomal sorting and signalling: an emerging role for sorting nexins. Nat Rev Mol Cell Biol. 2008; 9:574–582. [PubMed: 18523436]
4. Teasdale RD, Collins BM. Insights into the PX (phox-homology) domain and SNX (sorting nexin) protein families: structures, functions and roles in disease. Biochem J. 2012; 441:39–59. [PubMed: 22168438]
5. Pylypenko O, Lundmark R, Rasmuson E, Carlsson SR, Rak A. The PX-BAR membrane-remodeling unit of sorting nexin 9. EMBO J. 2007; 26:4788–4800. [PubMed: 17948057]
6. Pylypenko O, Ignatev A, Lundmark R, Rasmuson E, Carlsson SR, Rak A. A combinatorial approach to crystallization of PX-BAR unit of the human Sorting Nexin 9. J Struct Biol. 2008; 162:356–360. [PubMed: 18065239]
7. Wang Q, Kaan HY, Hooda RN, Goh SL, Sondermann H. Structure and plasticity of Endophilin and Sorting Nexin 9. Structure. 2008; 16:1574–1587. [PubMed: 18940612]
8. Lundmark R, Carlsson SR. SNX9 - a prelude to vesicle release. J Cell Sci. 2009; 122:5–11. [PubMed: 19092055]
9. Yarar D, Waterman-Storer CM, Schmid SL. SNX9 couples actin assembly to phosphoinositide signals and is required for membrane remodeling during endocytosis. Dev Cell. 2007; 13:43–56. [PubMed: 17609109]
10. Zhang J, Zhang X, Guo Y, Xu L, Pei D. Sorting nexin 33 induces mammalian cell micronucleated phenotype and actin polymerization by interacting with Wiskott-Aldrich syndrome protein. J Biol Chem. 2009; 284:21659–21669. [PubMed: 19487689]
11. Haberg K, Lundmark R, Carlsson SR. SNX18 is an SNX9 paralog that acts as a membrane tubulator in AP-1-positive endosomal trafficking. J Cell Sci. 2008; 121:1495–1505. [PubMed: 18411244]
12. Park J, Kim Y, Lee S, Park JJ, Park ZY, Sun W, Chang S. SNX18 shares a redundant role with SNX9 and modulates endocytic trafficking at the plasma membrane. J Cell Sci. 2010; 123:1742–1750. [PubMed: 20427313]
13. Badour K, McGavin MK, Zhang J, Freeman S, Vieira C, Filipp D, Siminovitch KA. Interaction of the Wiskott-Aldrich syndrome protein with sorting nexin 9 is required for CD28 endocytosis and cosignaling in T cells. Proc Natl Acad Sci U S A. 2007; 104:1593–1598. [PubMed: 17242350]
14. Shin N, Ahn N, Chang-Ileto B, Park J, Takei K, Ahn SG, Chang S. SNX9 regulates tubular invagination of the plasma membrane through interaction with actin cytoskeleton and dynamin 2. J Cell Sci. 2008; 121:1252–1263. [PubMed: 18388313]
15. Lundmark R, Carlsson SR. Sorting nexin 9 participates in clathrin-mediated endocytosis through interactions with the core components. J Biol Chem. 2003; 278:46772–46781. [PubMed: 12952949]
16. Yarar D, Surka MC, Leonard MC, Schmid SL. SNX9 activities are regulated by multiple phosphoinositides through both PX and BAR domains. Traffic. 2008; 9:133–146. [PubMed: 17988218]
17. van Weering JR, Verkade P, Cullen PJ. SNX-BAR proteins in phosphoinositide-mediated, tubular-based endosomal sorting. Semin Cell Dev Biol. 2010; 21:371–380. [PubMed: 19914387]

18. Posor Y, Eichhorn-Gruenig M, Puchkov D, Schoneberg J, Ullrich A, Lampe A, Haucke V. Spatiotemporal control of endocytosis by phosphatidylinositol-3,4-bisphosphate. Nature. 2013; 499:233–237. [PubMed: 23823722]
19. Beaty BT, Condeelis J. Digging a little deeper: the stages of invadopodium formation and maturation. Eur J Cell Biol. 2014; 93:438–444. [PubMed: 25113547]
20. Bendris N, Williams KC, Reis CR, Welf ES, Chen PH, Lemmers B, Schmid SL. SNX9 promotes metastasis by enhancing cancer cell invasion via differential regulation of RhoGTPases. Mol Biol Cell. 2016
21. Neumann S, Schmid SL. Dual role of BAR domain-containing proteins in regulating vesicle release catalyzed by the GTPase, dynamin-2. J Biol Chem. 2013; 288:25119–25128. [PubMed: 23861397]
22. Worby CA, Simonson-Leff N, Clemens JC, Huddler D Jr, Muda M, Dixon JE. Drosophila Ack targets its substrate, the sorting nexin DSH3PX1, to a protein complex involved in axonal guidance. J Biol Chem. 2002; 277:9422–9428. [PubMed: 11773052]
23. Gallop JL, Walrant A, Cantley LC, Kirschner MW. Phosphoinositides and membrane curvature switch the mode of actin polymerization via selective recruitment of toca-1 and Snx9. Proc Natl Acad Sci U S A. 2013; 110:7193–7198. [PubMed: 23589871]
24. Hicks L, Liu G, Ukken FP, Lu S, Bollinger KE, O'Connor-Giles K, Gonsalvez GB. Depletion or over-expression of Sh3px1 results in dramatic changes in cell morphology. Biol Open. 2015; 4:1448–1461. [PubMed: 26459243]
25. Lundmark R, Carlsson SR. Expression and properties of sorting nexin 9 in dynamin-mediated endocytosis. Methods Enzymol. 2005; 404:545–556. [PubMed: 16413299]
26. Soulet F, Yarar D, Leonard M, Schmid SL. SNX9 regulates dynamin assembly and is required for efficient clathrin-mediated endocytosis. Mol Biol Cell. 2005; 16:2058–2067. [PubMed: 15703209]
27. Doherty GJ, McMahon HT. Mechanisms of endocytosis. Annu Rev Biochem. 2009; 78:857–902. [PubMed: 19317650]
28. Lundmark R, Carlsson SR. The beta-appendages of the four adaptor-protein (AP) complexes: structure and binding properties, and identification of sorting nexin 9 as an accessory protein to AP-2. Biochem J. 2002; 362:597–607. [PubMed: 11879186]
29. Ramachandran R, Schmid SL. Real-time detection reveals that effectors couple dynamin's GTP-dependent conformational changes to the membrane. EMBO J. 2008; 27:27–37. [PubMed: 18079695]
30. Damke H, Binns DD, Ueda H, Schmid SL, Baba T. Dynamin GTPase domain mutants block endocytic vesicle formation at morphologically distinct stages. Mol Biol Cell. 2001; 12:2578–2589. [PubMed: 11553700]
31. Taylor MJ, Perrais D, Merrifield CJ. A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. PLoS Biol. 2011; 9:e1000604. [PubMed: 21445324]
32. Ma MP, Chircop M. SNX9, SNX18 and SNX33 are required for progression through and completion of mitosis. J Cell Sci. 2012
33. Shin N, Lee S, Ahn N, Kim SA, Ahn SG, YongPark Z, Chang S. Sorting nexin 9 interacts with dynamin 1 and N-WASP and coordinates synaptic vesicle endocytosis. J Biol Chem. 2007; 282:28939–28950. [PubMed: 17681954]
34. Mettlen M, Stoeber M, Loerke D, Antonescu CN, Danuser G, Schmid SL. Endocytic accessory proteins are functionally distinguished by their differential effects on the maturation of clathrin-coated pits. Mol Biol Cell. 2009; 20:3251–3260. [PubMed: 19458185]
35. Nunez D, Antonescu C, Mettlen M, Liu A, Schmid SL, Loerke D, Danuser G. Hotspots organize clathrin-mediated endocytosis by efficient recruitment and retention of nucleating resources. Traffic. 2011; 12:1868–1878. [PubMed: 21883765]
36. Chaudhary N, Gomez GA, Howes MT, Lo HP, McMahon KA, Rae JA, Parton RG. Endocytic crosstalk: cavins, caveolins, and caveolae regulate clathrin-independent endocytosis. PLoS Biol. 2014; 12:e1001832. [PubMed: 24714042]
37. Howes MT, Kirkham M, Riches J, Cortese K, Walser PJ, Simpson F, Parton RG. Clathrin-independent carriers form a high capacity endocytic sorting system at the leading edge of migrating cells. J Cell Biol. 2010; 190:675–691. [PubMed: 20713605]

38. Mayor S, Parton RG, Donaldson JG. Clathrin-independent pathways of endocytosis. Cold Spring Harb Perspect Biol. 2014; 6
39. Doherty GJ, Lundmark R. GRAF1-dependent endocytosis. Biochem Soc Trans. 2009; 37:1061–1065. [PubMed: 19754452]
40. Howard L, Nelson KK, Maciewicz RA, Blobel CP. Interaction of the metalloprotease disintegrins MDC9 and MDC15 with two SH3 domain-containing proteins, endophilin I and SH3PX1. J Biol Chem. 1999; 274:31693–31699. [PubMed: 10531379]
41. Seals DF, Courtneidge SA. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. Genes Dev. 2003; 17:7–30. [PubMed: 12514095]
42. Wu X, Gan B, Yoo Y, Guan JL. FAK-mediated src phosphorylation of endophilin A2 inhibits endocytosis of MT1-MMP and promotes ECM degradation. Dev Cell. 2005; 9:185–196. [PubMed: 16054026]
43. Renard HF, Simunovic M, Lemiere J, Boucrot E, Garcia-Castillo MD, Arumugam S, Johannes L. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. Nature. 2015; 517:493–496. [PubMed: 25517096]
44. Hoon JL, Wong WK, Koh CG. Functions and regulation of circular dorsal ruffles. Mol Cell Biol. 2012; 32:4246–4257. [PubMed: 22927640]
45. Wang JT, Kerr MC, Karunaratne S, Jeanes A, Yap AS, Teasdale RD. The SNX-PX-BAR family in macropinocytosis: the regulation of macropinosome formation by SNX-PX-BAR proteins. PLoS One. 2010; 5:e13763. [PubMed: 21048941]
46. Alto NM, Weflen AW, Rardin MJ, Yarar D, Lazar CS, Tonikian R, Dixon JE. The type III effector EspF coordinates membrane trafficking by the spatiotemporal activation of two eukaryotic signaling pathways. J Cell Biol. 2007; 178:1265–1278. [PubMed: 17893247]
47. Piscatelli HL, Li M, Zhou D. Dual 4- and 5-phosphatase activities regulate SopB-dependent phosphoinositide dynamics to promote bacterial entry. Cell Microbiol. 2015
48. Weflen AW, Alto NM, Viswanathan VK, Hecht G. E. coli secreted protein F promotes EPEC invasion of intestinal epithelial cells via an SNX9-dependent mechanism. Cell Microbiol. 2010; 12:919–929. [PubMed: 20088948]
49. Holmes A, Muhlen S, Roe AJ, Dean P. The EspF effector, a bacterial pathogen's Swiss army knife. Infect Immun. 2010; 78:4445–4453. [PubMed: 20679436]
50. Yamaguchi H, Condeelis J. Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim Biophys Acta. 2007; 1773:642–652. [PubMed: 16926057]
51. Lohmer LL, Kelley LC, Hagedorn EJ, Sherwood DR. Invadopodia and basement membrane invasion in vivo. Cell Adh Migr. 2014; 8:246–255. [PubMed: 24717190]
52. Bendris N, Stearns CJ, Reis CR, Rodriguez-Canales J, Liu H, Witkiewicz AW, Schmid SL. Sorting nexin 9 negatively regulates invadopodia formation and function in cancer cells. J Cell Sci. 2016; 129:2804–2816. [PubMed: 27278018]
53. Stearns, CJS. Characterizing the interplay between FAK, SRC and ACK2 in the regulation of SH3PX1 activity. Cornell University; 2007.
54. Ma MP, Robinson PJ, Chircop M. Sorting nexin 9 recruits clathrin heavy chain to the mitotic spindle for chromosome alignment and segregation. PLoS One. 2013; 8:e68387. [PubMed: 23861900]
55. Royle SJ, Bright NA, Lagnado L. Clathrin is required for the function of the mitotic spindle. Nature. 2005; 434:1152–1157. [PubMed: 15858577]
56. Almendinger J, Doukoumetzidis K, Kinchen JM, Kaech A, Ravichandran KS, Hengartner MO. A conserved role for SNX9-family members in the regulation of phagosome maturation during engulfment of apoptotic cells. PLoS One. 2011; 6:e18325. [PubMed: 21494661]
57. Lu N, Shen Q, Mahoney TR, Liu X, Zhou Z. Three sorting nexins drive the degradation of apoptotic cells in response to PtdIns(3)P signaling. Mol Biol Cell. 2011; 22:354–374. [PubMed: 21148288]
58. Worby CA, Simonson-Leff N, Clemens JC, Kruger RP, Muda M, Dixon JE. The sorting nexin, DSH3PX1, connects the axonal guidance receptor, Dscam, to the actin cytoskeleton. J Biol Chem. 2001; 276:41782–41789. [PubMed: 11546816]

59. Knaevelsrud H, Carlsson SR, Simonsen A. SNX18 tubulates recycling endosomes for autophagosome biogenesis. Autophagy. 2013; 9:1639–1641. [PubMed: 24113029]
60. Knaevelsrud H, Soreng K, Raiborg C, Haberg K, Rasmuson F, Brech A, Simonsen A. Membrane remodeling by the PX-BAR protein SNX18 promotes autophagosome formation. J Cell Biol. 2013; 202:331–349. [PubMed: 23878278]
61. Nandez R, Balkin DM, Messa M, Liang L, Paradise S, Czapla H, De Camilli P. A role of OCRL in clathrin-coated pit dynamics and uncoating revealed by studies of Lowe syndrome cells. Elife. 2014; 3:e02975. [PubMed: 25107275]
62. Ezernitchi AV, Vaknin I, Cohen-Daniel L, Levy O, Manaster E, Halabi A, Baniyash M. TCR zeta down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. J Immunol. 2006; 177:4763–4772. [PubMed: 16982917]
63. Ish-Shalom E, Meirow Y, Sade-Feldman M, Kanterman J, Wang L, Mizrahi O, Baniyash M. Impaired SNX9 Expression in Immune Cells during Chronic Inflammation: Prognostic and Diagnostic Implications. J Immunol. 2016; 196:156–167. [PubMed: 26608909]
64. Bergsmedh A, Szeles A, Henriksson M, Bratt A, Folkman MJ, Spetz AL, Holmgren L. Horizontal transfer of oncogenes by uptake of apoptotic bodies. Proc Natl Acad Sci U S A. 2001; 98:6407–6411. [PubMed: 11353826]
65. Wang X, Yang C. Programmed cell death and clearance of cell corpses in Caenorhabditis elegans. Cell Mol Life Sci. 2016
66. Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol. 2002; 2:965–975. [PubMed: 12461569]
67. Lundmark R, Carlsson SR. Regulated membrane recruitment of dynamin-2 mediated by sorting nexin 9. J Biol Chem. 2004; 279:42694–42702. [PubMed: 15299020]
68. Yeow-Fong L, Lim L, Manser E. SNX9 as an adaptor for linking synaptojanin-1 to the Cdc42 effector ACK1. FEBS Lett. 2005; 579:5040–5048. [PubMed: 16137687]
69. Miele AE, Watson PJ, Evans PR, Traub LM, Owen DJ. Two distinct interaction motifs in amphiphysin bind two independent sites on the clathrin terminal domain beta-propeller. Nat Struct Mol Biol. 2004; 11:242–248. [PubMed: 14981508]
70. Baumann C, Lindholm CK, Rimoldi D, Levy F. The E3 ubiquitin ligase Itch regulates sorting nexin 9 through an unconventional substrate recognition domain. FEBS J. 2010; 277:2803–2814. [PubMed: 20491914]
71. Schulze WX, Mann M. A novel proteomic screen for peptide-protein interactions. J Biol Chem. 2004; 279:10756–10764. [PubMed: 14679214]
72. Lin Q, Lo CG, Cerione RA, Yang W. The Cdc42 target ACK2 interacts with sorting nexin 9 (SH3PX1) to regulate epidermal growth factor receptor degradation. J Biol Chem. 2002; 277:10134–10138. [PubMed: 11799118]
73. Marches O, Batchelor M, Shaw RK, Patel A, Cummings N, Nagai T, Frankel G. EspF of enteropathogenic Escherichia coli binds sorting nexin 9. J Bacteriol. 2006; 188:3110–3115. [PubMed: 16585770]
74. Tahoun A, Siszler G, Spears K, McAteer S, Tree J, Paxton E, Mahajan A. Comparative analysis of EspF variants in inhibition of Escherichia coli phagocytosis by macrophages and inhibition of E. coli translocation through human- and bovine-derived M cells. Infect Immun. 2011; 79:4716–4729. [PubMed: 21875965]
75. Rangarajan ES, Park H, Fortin E, Sygusch J, Izard T. Mechanism of aldolase control of sorting nexin 9 function in endocytosis. J Biol Chem. 2010; 285:11983–11990. [PubMed: 20129922]
76. MaCaulay SL, Stoichevska V, Grusovin J, Gough KH, Castelli LA, Ward CW. Insulin stimulates movement of sorting nexin 9 between cellular compartments: a putative role mediating cell surface receptor expression and insulin action. Biochem J. 2003; 376:123–134. [PubMed: 12917015]
77. Sowa ME, Bennett EJ, Gygi SP, Harper JW. Defining the human deubiquitinating enzyme interaction landscape. Cell. 2009; 138:389–403. [PubMed: 19615732]
78. Ukken FP, Bruckner JJ, Weir KL, Hope SJ, Sison SL, Birschbach RM, O'Connor-Giles KM. BAR-SH3 sorting nexins are conserved interacting proteins of Nervous wreck that organize synapses and promote neurotransmission. J Cell Sci. 2016; 129:166–177. [PubMed: 26567222]

Trends

Migration or cell division requires the spatial and temporal coordination of vesicular trafficking, membrane remodeling, signaling, and cytoskeletal dynamics that are orchestrated by protein scaffolds.

The multidomain scaffold SNX9 is a major dynamin binding partner that binds and activates N-WASP to regulate actin assembly. It is well known for its role in clathrin-mediated endocytosis.

The scaffolding activity of SNX9 and its interactions with functionally diverse proteins have roles in clathrin-independent endocytosis, cell migration and invasion, mitosis, and cytokinesis.

Changes in SNX9 expression levels are associated with human disease, including cancer. Low levels of SNX9 promote formation and activity of invadosomes, whereas high levels promote cell migration through collagen. This bimodal function is reflected in changes in SNX9 proteins levels during tumor progression and metastasis.

Outstanding questions

- Do all of SNX9's diverse cellular functions require membrane interactions and involve membrane remodeling or curvature sensing?
- How are the many diverse protein interactions with SNX9 spatially and temporally regulated?
- How does SNX9 regulate Rho-family GTPases? Are GTPases from other families such as Rab or Arf targeted as well?
- How does phosphorylation by ACK, Src, FAK or other kinases alter SNX9 interactions and functions? What are the kinases and their phosphorylation sites?
- Are alterations in the activity of SNX9 associated with overexpression or activation of Src, FAK and ACK kinases in cancer?
- Besides phosphorylation, how is SNX9 regulated (i.e. posttranslational modifications, gene expression, or microRNAs)?
- How functionally divergent are SNX9 family members? How different are their biochemical properties and interactomes?

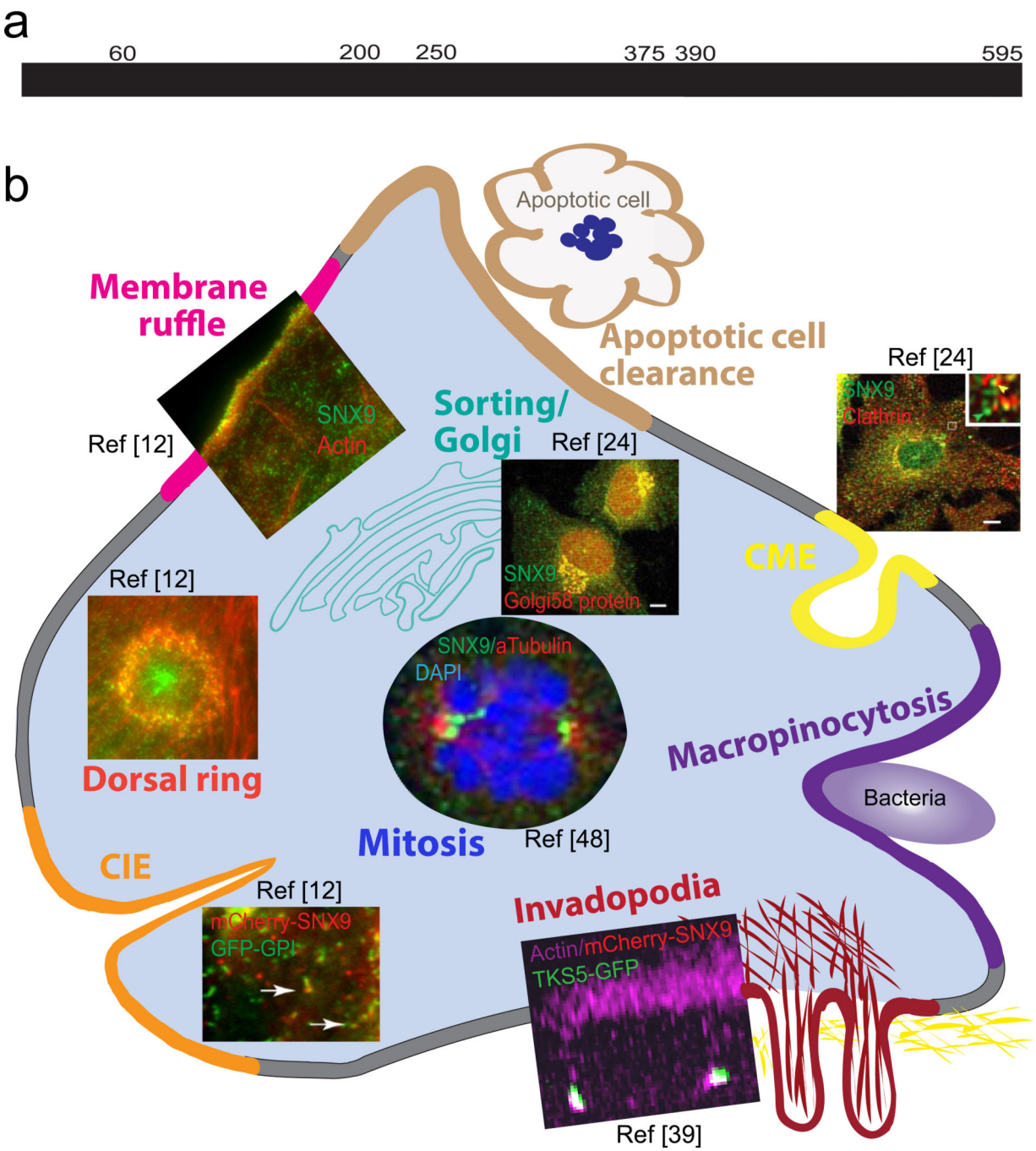

Figure 1. Domain structure and subcellular localization of SNX9

A. SNX9 is a multi-domain protein. The N-terminal SH3 domain is followed by a low complexity (LC) region, both are protein interaction domains. The PX and BAR domains form a functional module for membrane interactions. **B.** The diverse subcellular localizations of SNX9 reflect is multifunctionality. At the plasma membrane, SNX9 colocalizes with clathrin [26], GPI-anchored proteins [16], lamellapodia and dorsal rings [16], reflecting its role in clathrin-dependent and -independent endocytic mechanisms. SNX9 is also localizes to the Golgi apparatus [26], where it may function in transport of

ADAM precursors [40] or GM130 [32] from the Golgi, to invadopodia [52] and to mitotic spindles [54], consistent with its role as a multifunctional scaffold involved in multiple cellular processes.

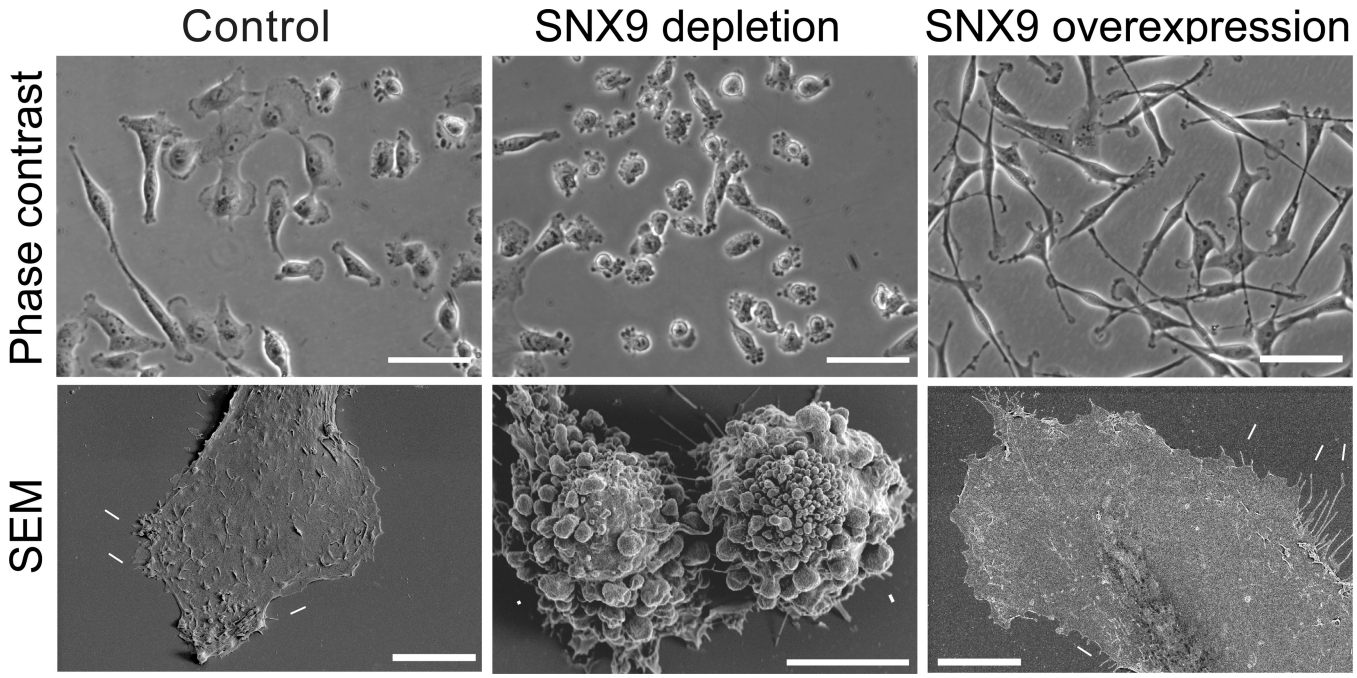

Figure 2. Differential expression of SNX9 affects cell morphology of human-derived cancer cells

Phase contrast and scanning electron (SEM) micrographs of MDA-MB-231 breast cancer cells either untreated (Control), depleted (siRNA-mediated knockdown) or overexpressing SNX9. Arrows indicate filopodia in control and SNX9 overexpression conditions. Arrowheads indicate blebs in SNX9 depletion. Bars: 100μm (Phase) and 10μm (SEM).

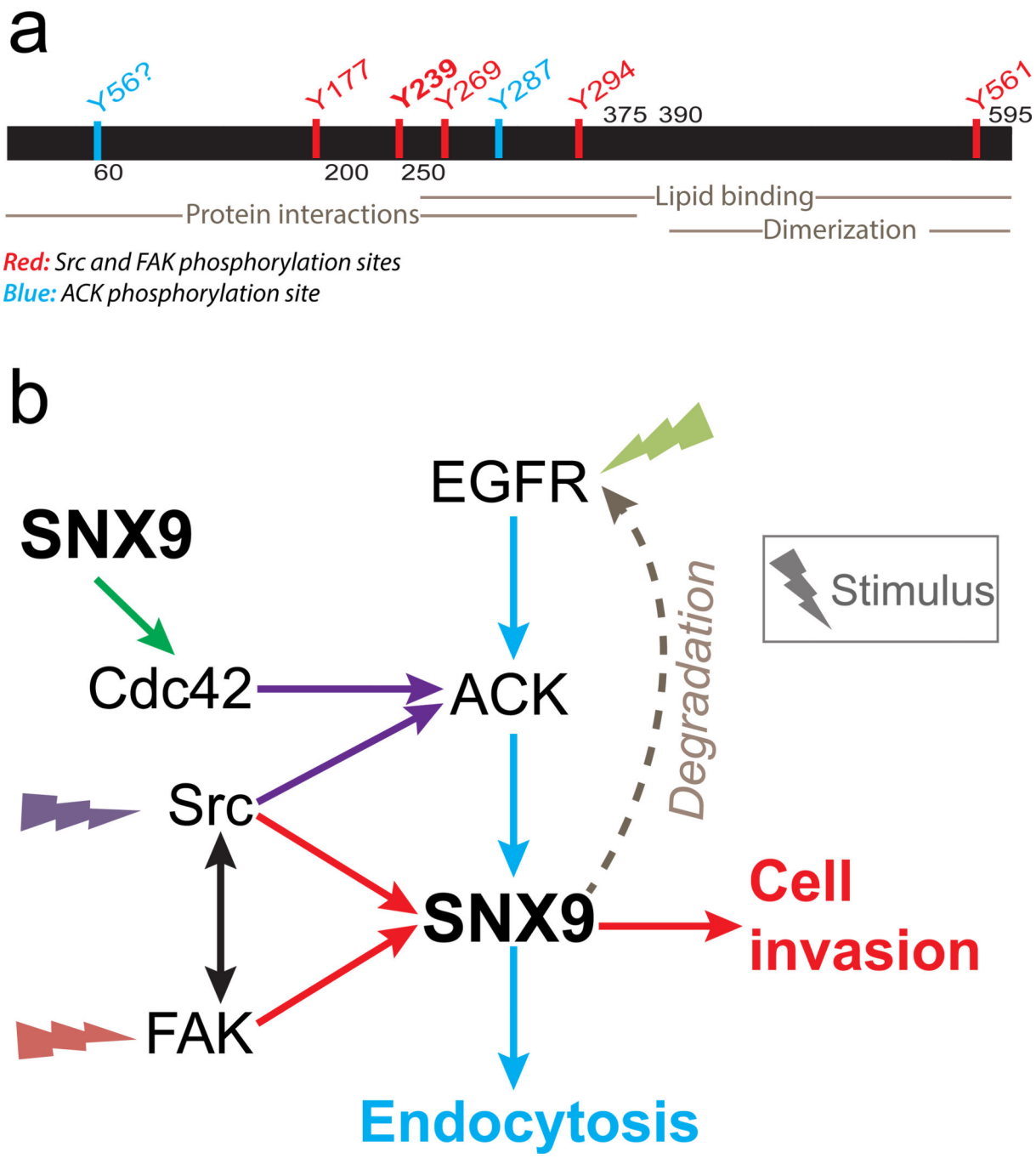

Figure 3. SNX9 is regulated by phosphorylation

A. SNX9 has been identified as a target for three tyrosine kinases. Src and FAK kinases share the same phosphorylation sites (red), with Y239 as the main phosphorylated residue. ACK phosphorylates Y287 on SNX9 in mammalian cells. By homology with Drosophila SNX9, Y56 is potentially phosphorylated by ACK in mammalian cells (ACK phosphorylation sites in blue). **B.** Multiple kinases integrate various stimuli to regulate different cellular functions through SNX9. Upon EGF stimulation, ACK phosphorylates SNX9 and regulates its function in endocytosis. In return, SNX9 enhances EGFR

degradation. Src/FAK phosphorylate SNX9 on multiple sites to modulate its function in cell invasion. Note that, ACK is also directly activated by cdc42 and Src. Thus, SNX9's scaffolding activity can be modulated in response to extracellular signals.

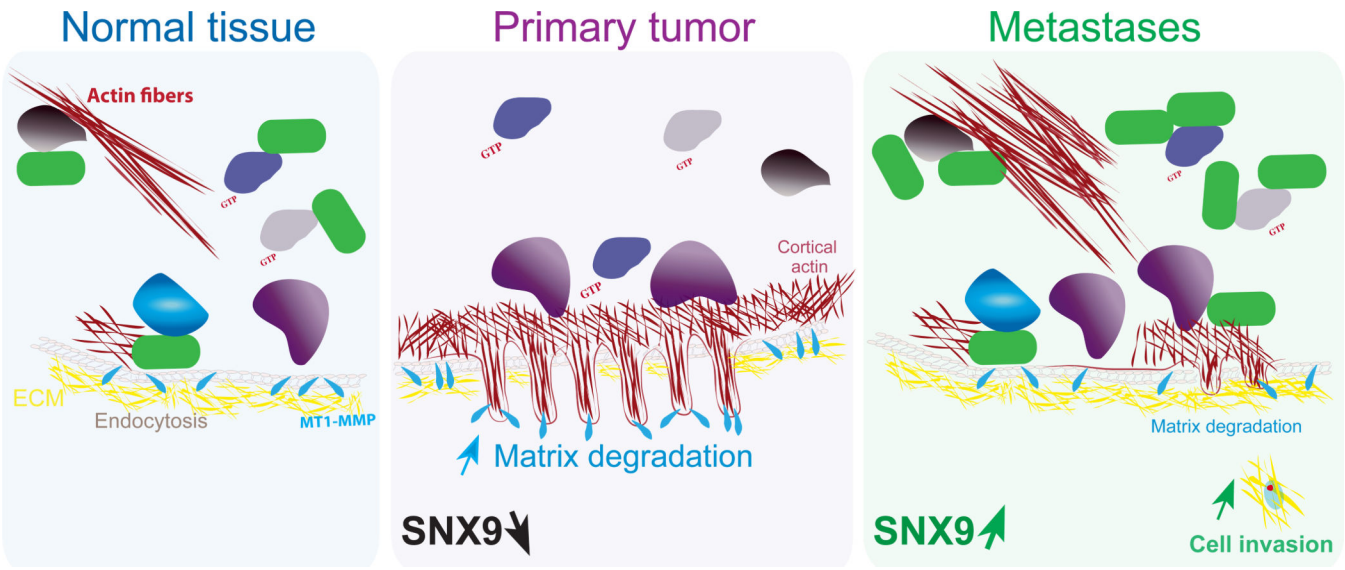

Figure 4. A dual role for SNX9 in cancer

SNX9 is a scaffold protein that directly binds to the clathrin-mediated endocytosis (CME) regulator, dynamin. In parallel, SNX9 also interacts with proteins that are either directly or indirectly involved in actin polymerization or remodeling, namely N-WASP and the GTPases, RhoA and Cdc42. While SNX9 binds to N-WASP and to dynamin *via* its SH3 domain, and to TKS5 more likely *via* its LC domain, it is not known which domain binds to RhoA and Cdc42. In primary tumors, SNX9 protein expression decreases, leading to changes in GTP-loading of the Rho-family GTPases, RhoA and cdc42. Lower expression levels of SNX9 enhance the formation and subsequent degradative activity of invadopodia by enhancing recruitment of the maturation marker, TKS5, and possibly *via* increased RhoA activation. Clathrin-indepenent endocytosis is also decreased leading to the accumulation of the ECM protease MT1-MMP at the tips of invadopodia. In metastases, higher SNX9 protein expression levels lead to increased actin fiber formation via N-WASP and activated Cdc42. Additionally, SNX9 binds to TKS5 and impairs invadopodia formation and function. Data suggests that bimodal changes in SNX9 expression levels can switch cancer cell phenotypes. Under conditions of low SNX9 expression, cells display an active invadopodial-ECM degradation phenotype favoring local spread of tumor cells from aggressive primary tumors. Under conditions of high SNX9 expression, the cells convert to a more invasive and metastatic phenotype that is less dependent of invadopodia.

Table 1

Binding partners of SNX9

Binding partner	Pathway	SNX9 domain	Reference
Dynamin	Endocytosis	SH3	[15,26,67]
N-WASP	Actin cytoskeleton	SH3	[9,13,14,33]
ADAM 9/15	Proteases at PM	SH3	[40]
DOCK1 (NCK1 in mammals)	Adaptor protein	SH3	[58]
Synaptojanin-I	Endocytosis	SH3	[68,69]
ITCH ubiquitin ligase	Ubiquitinylation	SH3	[70]
Son of sevenless 1 (Sos1) and Sos2	Regulators of Ras	SH3	[71]
Cdc42-associated kinase (ACK)	Endocytosis Signaling	SH3	[68,72]
EspF	Bacterial protein	SH3	[46,73,74]
Clathrin	Endocytosis	LC	[15]
AP2	Endocytosis	LC	[15,28]
Aldolase	Metabolism	LC	[67,75]
ARP2/3	Actin cytoskeleton	LC	[14]
TKS5	Adaptor protein Invadopodia	LC	[52]
PI3K p85	Signaling	PX	[13]
PtdIns(4)P-5-kinases Iα, Iβ and Iγ	Signaling	PX	[14]
RhoA	Actin cytoskeleton/signaling	?	[20]
Cdc42	Actin cytoskeleton/signaling	?	[20]
Src	Signaling	?	[20]
Insulin receptor	Signaling	?	[76]
Herpes Virus-Associated Ubiquitin Specific Protease 7 (USP7)	Deubiquitination	?	[77]
Ubiquitin Specific Protease 25 (USP25)	Deubiquitination	?	[77]
Poly (ADP-Ribose) Polymerase Family, Member 11 (PARP11)	DNA repair Apoptosis	?	[77]
TL132	Homologous to deubiquitinating enzymes	?	[77]
CD247 (subunit of TCR)	T cell activation	?	[63]
Nervous wreck protein (NWK)	Neuromuscular junctions	?	[78]
Oculocerebrorenal syndrome of Lowe	Lipid phosphatase	?	[61]

Binding partner	Pathway	SNX9 domain	Reference
protein (OCRL)			