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MINI-REVIEW

The role of Exo70 in exocytosis and beyond

Yueyao Zhu, Bin Wu, and Wei Guo

Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

ABSTRACT

The exocyst complex mediates the tethering of secretory vesicles to the plasma membrane before SNARE-mediated membrane fusion. Recent studies have implicated the exocyst in a wide range of cellular processes. Particularly, research on the Exo70 subunit of the complex has linked the function of the exocyst in exocytosis to cell adhesion, migration and invasion. In this review, we will discuss the recent work on how Exo70 regulates these cellular processes, and how small GTPases and kinases interact with Exo70 to orchestrate its function in exocytosis and cytoskeleton organization. The study of Exo70 contributes to the understanding of many pathophysiological processes from organogenesis to cancer metastasis.

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The exocyst is an octameric protein complex consisting of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84. It primarily functions in the tethering of secretory vesicles to the plasma membrane before the soluble N-ethylmaleimide–sensitive factor attachment protein receptors (SNARE)-mediated membrane fusion.[1,2] Recent studies from different fields have implicated the exocyst in various pathophysiological processes, such as cell cycle progression, tumor invasion and primary ciliogenesis.[1,3] Particularly, study of the Exo70 subunit of the complex has unveiled some new mechanisms that link exocytosis to cell adhesion, migration and tumor invasion. Exo70, together with Sec3, mediates the association of the exocyst complex to the plasma membrane, which is a critical step for vesicle tethering.[4,5] Exo70 directly interacts with $PI(4,5)P_2$ in the plasma membrane through several negatively charged residues at its C-terminus. Disrupting the association of Exo70 and Sec3 with the plasma membrane results in defects in exocytosis. While the role of Exo70 in exocytosis has been well-established, we highlight the other cellular processes that implicate this interesting protein.

Exo70 in cell adhesion

Cells are connected to their neighboring cells or attached to the extracellular matrix (ECM). These adhesive interactions are important for maintaining cellular functions including proliferation and survival. Recent evidence has shown that Exo70 contributes to cell-ECM interaction. Exo70 is implicated in the targeting of Caveolin-1-positive vesicles to the plasma membrane during cell re-attachment to ECM (Fig. 1).[6] Caveolin-1 is a major component of caveolae, which are believed to modulate the cell interaction with ECM through the association with integrin-mediated adhesion.[7] The depletion of Exo70 impaired the delivery of Caveolin-1-positive vesicles to plasma membrane and consequently inhibited cell spreading.[6] Similar phenotype was observed by silencing Sec5, another component of exocyst, in MEFs.[8] In addition, Sec5 has also been implicated in focal complex formation.[9] These studies suggest that Exo70 coordinates with other exocyst components in this function.

In epithelial cells, the exocyst has been implicated in the formation of junctions.[10-14] It was shown that Exo70 mediates the directional transport of E-Cadherin to lateral membrane and promotes the clustering of E-Cadherin on plasma membrane during the formation of adherens junctions (Fig. 1).[15] Immunofluorescence microscopy studies indicated that lack of Exo70 led to an abnormal distribution of E-Cadherin on the lateral membrane and impaired maturation of adherens junction. Interestingly, Exo70 has several splicing isoforms that are differentially expressed in epithelial cells ("Exo70-E") and mesenchymal cells ("Exo70-M"), respectively. Expression of Exo70-E contributes to epithelia formation and mesenchymal-epithelial transition.[16]

CONTACT Wei Guo guowei@sas.upenn.edu 433 S. University Ave. Carolyn Lynch Laboratory, Department of Biology, University of Pennsylvania, Philadelphia, PA, USA.

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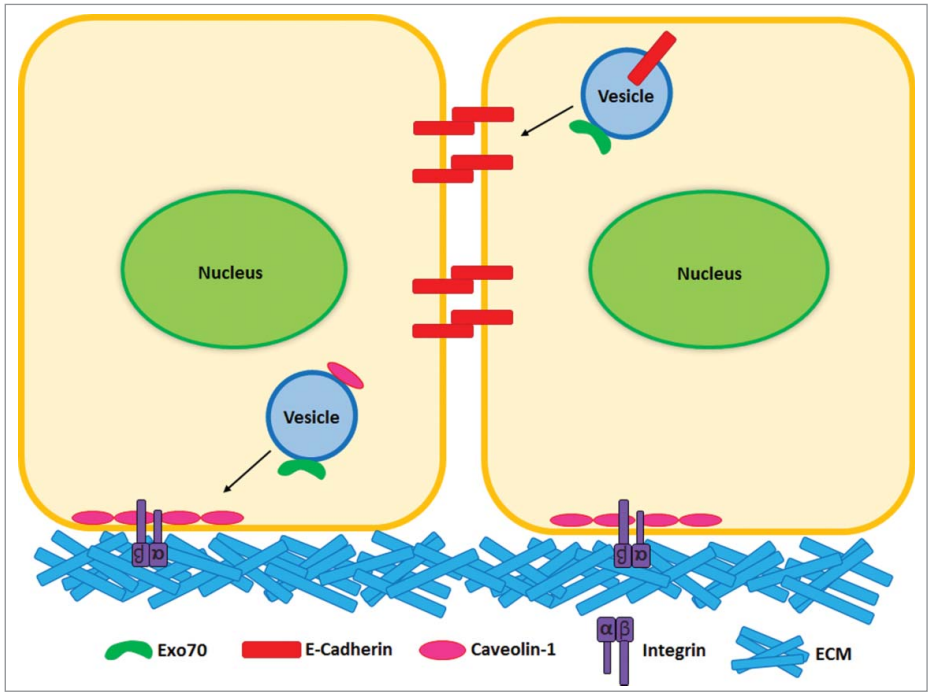

Figure 1. Exo70 in cell-ECM and cell-cell interaction. Exo70 targets integrin- and Caveolin-1-positive vesicles to the plasma membrane during cell attachment to ECM. Exo70 also directs the transport of E-Cadherin-positive vesicles to the lateral membrane to promote the formation of adherens junction.

Exo70 in cell migration and invasion

Cell migration involves actin network reorganization, membrane remodeling, and trafficking of signaling and adhesion proteins to the leading edge. Actin polymerization and branching is controlled by the Arp2/3 complex.[17] Exo70 directly binds to the Arp2/3 complex, and is localized to the leading edge of migrating cell.[18,19] Exo70 enhances the interaction of the Arp2/3 complex with WAVE2 and accelerates actin branching (Fig. 2). This process is likely to be independent of the holo-exocyst complex as the stimulatory effect can be detected *in vitro* with recombinant Exo70.[19] It was recently reported that Exo70 is also involved in the interaction of the exocyst complex and the WAVE regulatory complex (WRC).[20] The WRC regulates the dynamics of actin cytoskeleton by stimulating the activity of the Arp2/3 complex at the plasma membrane to control cell motility. The interaction between Exo70 and WRC is responsible for the recruitment of WRC to sites of the plasma membrane where protrusions are formed in migrating cells. WRC recruitment coincides with leading edge movement, demonstrating that the association of Exo70 with WRC contributes to cell motility. It is very likely that Exo70 plays dual roles in migrating cells through mediating actin cytoskeleton: a kinetic activator that directly controls actin branching and a molecular carrier that transports regulatory molecules of Arp2/3 complex. In addition to remodeling actin, Exo70 can form oligomers via its N-terminus to generate negative curvature on the plasma membrane, a process that could also be independent of the whole exocyst complex.[21] This membrane-deforming ability contributes to protrusion formation (Fig. 2). As elaborated above, the exocyst also mediates the delivery of focal adhesion molecules such as integrins to the plasma membrane. In addition, Exo70 was shown to interact with PIPKIγi2 to direct the polarized integrin trafficking during directional cell migration.[22]

Cell invasion often involves the generation of matrix-degrading structures that are termed as invadopodia. Mueller and colleagues proposed a sequential model of invadopodia formation, which starts with actin-based structure precursor and then mature into ECM degradation.[23] Inhibition of invadopodial structure assembly blocks the ECM degradation. With RNAi knockdown of the Exo70, the number of invadopodial sites, as represented by actin puncta, diminishes, and ECM degradation is reduced significantly.[24] In addition to modulating the Arp2/3 complex-mediated actin remodeling, Exo70 regulates the invadopodial activity by promoting MMPs secretion (Fig. 2),[24] Gelatin zymography detected a significant reduction in the levels of MMP-2 and MMP-9 in Exo70 knockdown cells, whereas re-expression of RNAi-resistant Exo70 recovers the secretion of the two proteins.

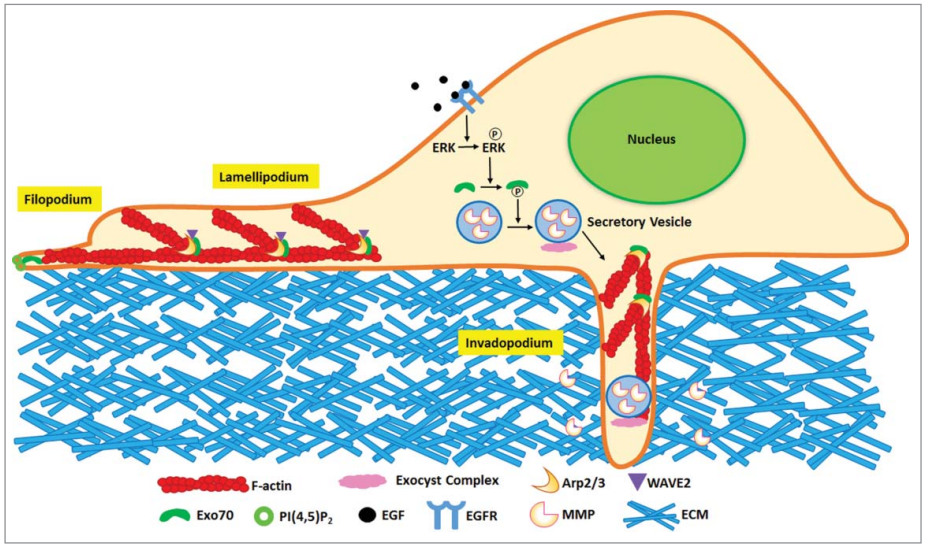

Figure 2. Exo70 in cell migration and invasion. During cell migration, Exo70 generates membrane curvature, which contributes to membrane protrusion at the leading edge. Exo70 also promotes the interaction of the Arp2/3 complex with its activators such as WAVE2, stimulating actin filament nucleation and branching. During cell invasion, Exo70, together with other exocyst components, participates in the secretion of matrix metalloproteinases (MMPs), which mediate ECM degradation by invadopodia. Phosphorylation of Exo70 by ERK promotes the assembly of Exo70 with other exocyst components, thereby enhancing MMP exocytosis.

Regulation of Exo70

The Rho family of small GTPases regulates many cellular processes. In yeast, Exo70 was identified as downstream effector of Rho3 and Cdc42, both of which were implicated in regulating polarized exocytosis.[25-27] In mammalian cells, Exo70 is associated with TC10, another Rho family protein. In response to insulin, GTP-TC10 recruits Exo70 and other members of the exocyst, which tethers Glut4-containing vesicles to the plasma membrane.[28] In developing neurons, NGF induces TC10-Exo70 complex assembly and this complex locally prevents Cdc42-dependent activation of N-WASP at the plasma membrane.[29] Additionally, TC10-Exo70 complex stimulated by IGF functions in axonal membrane expansion and polarized delivery of IGF-1 receptor.[30]

Recent studies underlie the involvement of MAPK signaling in the secretory pathway. Exo70 was identified as a direct substrate of ERK1/2.[31] Upon EGF stimulation, ERK1/2 phosphorylates Exo70 at serine 250. The phosphorylation of Exo70 promotes the assembly of the exocyst complex, which regulates MMP exocytosis and invadopodia activity (Fig. 2). It was also found that Exo70 is highly phosphorylated in metastatic melanoma cells from patients with $BRAF^{V600E}$ mutation; inhibition of the *RAF-MEK-ERK* signaling pathway decreases the phosphorylation of Exo70 and inhibited invadopodia formation in melanoma cells.[32]

Conclusions and perspectives

In this mini-review, we summarize the recent studies regarding the function of Exo70 in cells. Through the targeting of cargos to specific membrane domains, and through its involvement in membrane curvature induction and actin remodeling, Exo70 plays important roles in many cellular processes.

Despite these progresses, many questions remain unanswered. For example, as Exo70 was shown to interact with many molecules, how are these interactions spatially and temporally orchestrated for certain cellular functions? Also, since there are so many isoforms for Exo70,[32,33] what controls the expression of these isoforms in cells, in different tissues, and at different developmental stages? These questions are much better addressed in plant biology,[33] but still need to be studied in depth in mammalian cells. Answering these questions will not only elucidate the function of Exo70 and the exocyst complex, but also contribute to the understanding of many pathophysiological processes from organogenesis to cancer metastasis.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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