

REVIEW ARTICLE

Cell polarity during motile processes: keeping on track with the exocyst complex

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Motile processes are critical for several physiological and pathological situations such as embryonic development, tumour dissemination and metastasis. Migrating cells, or developing neurons, need to establish front–rear polarity consisting of actin-driven extension of the leading edge and traffic of components that are essential for membrane extension and cell adhesion at the front. Previously, several studies have suggested that the exocyst complex is critical for the establishment and maintenance of cell polarity. This octameric complex controls the docking and insertion of exocytic vesicles to growing areas of the plasma membrane. The aim of the present review is to detail recent

advances concerning the molecular and structural organization of the exocyst complex that help to elucidate its role in cell polarity. We will also review the function of the exocyst complex and some of its key interacting partners [including the small GTP-binding protein Ral, aPKCs (atypical protein kinase Cs) and proteins involved in actin assembly] in the formation of plasma extensions at the leading edge, growth cone formation during axonal extension and generation of cell movement.

Key words: actin cytoskeleton, exocyst complex, invasion, motility, neuronal development, small GTPase.

INTRODUCTION

In *Saccharomyces cerevisiae*, where the complex was first identified, the exocyst complex comprises eight subunits named Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 [1]. Genetic approaches revealed that yeast strains carrying mutant exocyst alleles accumulated cytoplasmic transport vesicles and showed defects in polarized secretion to the growing bud (Figure 1A). These, and other, observations led to the hypothesis that the exocyst complex functions primarily during polarized secretion by controlling the docking of intracellular vesicles to specific exocytic sites at the PM (plasma membrane) [2]. The exocyst complex also exists in mammals, although amino acid conservation between yeast and mammalian subunits is low and does not extend above 20–30% identity [3,4]. The mammalian subunits are called EXOCs (exocyst complex components) 1–8, however, for clarity we will keep the yeast nomenclature herein. In mammalian cells, the exocyst complex is also essential for diverse cellular processes requiring polarization of membrane trafficking such as in developing neurons, where the exocyst is localized to the tips of neurites and is required for neurite outgrowth [5]; or in epithelial cells, in which the exocyst complex accumulates at the level of the adherens junction in a region of active membrane addition [6,7]; and in migrating cells (Figure 1) [8,9].

In the present review, we first discuss our understanding of the exocyst complex at a molecular and structural level. Then, we detail the specific role(s) of the exocyst complex and its network of interacting partners in the spatial and temporal control of polarized exocytosis during motile processes.

TOWARDS A STRUCTURAL UNDERSTANDING OF EXOCYST-MEDIATED VESICLE DOCKING

The structural characterization of high-molecular-mass complexes is always a technical challenge. The native purified mammalian exocyst complex with its ~700 kDa molecular mass represents a huge object for X-ray crystallography analysis. Correct folding of each subunit is generally interdependent in such macromolecular assemblies, leading to difficulty with purification schemes and the instability of isolated subunits. However, using limited proteolysis and secondary-structure prediction, some stable fragments could be crystallized allowing the resolution of the structure of several subunits or subunit domains (Figure 2 and Table 1) [10].

At a first glance, the most striking observation is that, despite low sequence homologies between different subunits, overall the C-terminal region of Sec6, Sec15 and Exo84 and full-length Exo70 share the same novel fold (Figure 2) [11–13]. These domains are made of compact helical-bundle repeats connected by flexible linkers forming an elongated rod [14]. Interestingly, other complexes with known vesicle-tethering function [Dsl1p, GARP (Golgi-associated retrograde protein) complex and COG (conserved oligomeric Golgi) complex] also adopt the structural organization of the exocyst complex, establishing a structural relationship between several multi-subunit-tethering complexes and implying a putative common progenitor [10,15,16].

The structure of the N-terminal region of Sec5 and Exo84 was solved in complex with the small GTPase RalA, in its active GTP-bound state [17,18]. Indeed, Sec5 and Exo84 interact with

Abbreviations used: aPKC, atypical protein kinase C; EM, electron microscopy; CAV1, caveolin-1; Cdc42, cell division cycle 42; ERK, extracellular-signal-regulated kinase; IQGAP, IQ motif containing GTPase-activating protein 1; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; NRK, normal rat kidney; PH, pleckstrin homology; PIP₂, phosphatidylinositol 4,5-bisphosphate; PM, plasma membrane; RBD, Ral-binding domain; SNARE, soluble N-ethylmaleimide sensitive factor attachment protein receptor; VASP, vasodilator-stimulated phosphoprotein; VAMP, vesicle-associated membrane protein.

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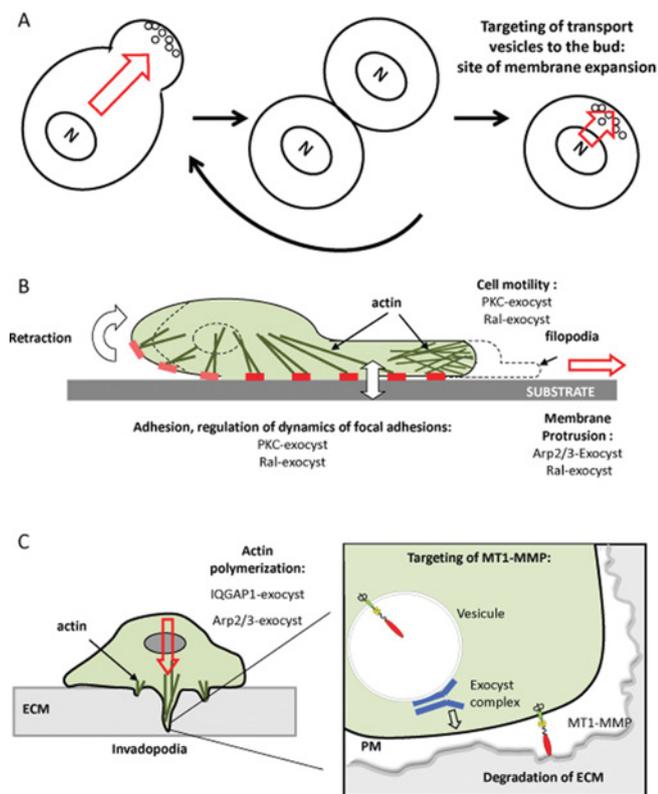


Figure 1 The role of the exocyst complex and its partners in cell polarity

The exocyst localizes to sites of active secretion: **(A)** at the growing bud during fission of budding yeast; **(B)** at the leading edge during cell motility; and **(C)** at the invadopodia during ECM (extracellular matrix) degradation. MT1-MMP, membrane type 1 MMP; N, nucleus.

RalA-GTP, and hence they were both shown to act as downstream RalA effectors [17,18]. The crystal structure of the RBD (Ral-binding domain) of Exo84 in complex with RalA-GTP revealed a putative PH (pleckstrin homology) domain fold. PH domains are known to interact with phospholipids [19]. However, whether

Table 1 Summary of published exocyst subunit crystallographic structures

Subunit (residues)	Species	PDB code	Reference(s)
Sec3 (71–241)	Yeast	3HIE	[23]
Sec3 (1–320)	Yeast	3A58	[24]
Sec5 (1–99)	<i>Rattus norvegicus</i>	1UAD	[18]
Sec6 (411–805)	Yeast	2FJI	[11]
Exo70 (62–623 and 58–543)	Yeast	2B1E and 2B7M	[13,25]
Exo70 (85–653)	Mouse	2PFT	[88]
Sec15 (1–325)	<i>Drosophila</i>	2A2F	[12]
Exo84 (9–113)	<i>Rattus norvegicus</i>	1ZC3	[17]
Exo84 C-terminal (523–753)	Yeast	2D2S	[13]

Exo84 can interact with lipids has never been reported and this should be formally addressed for a complete understanding of the interaction between the exocyst complex and membranes.

In the case of Sec5, the surface of interaction with RalA consists of a continuous antiparallel β -sheet, which had been previously found in Ras and Rap1 downstream effectors [18,20–22]. It is worth noting that the RBD of Exo84 and Sec5 share a common surface of interaction on RalA-GTP and their binding is mutually exclusive [17].

Recently, the structure of the N-terminal region of yeast Sec3 was resolved, both in complex with the small GTPase Rho1 and alone, revealing a cryptic PH domain in the Sec3 subunit that represents a new PH domain subclass [23,24]. These structures allowed the identification of a patch of basic residues that may interact with phospholipids. Targeting of yeast Sec3 to the PM is mediated both through direct binding to lipids based on this basic patch and via an interaction with membrane-anchored Rho1-GTP [24]. This is in contrast with mammalian Sec3 that lost the N-terminal Rho-binding domain and patch of basic residues, suggesting different exocyst and lipid interplay in yeast and mammals.

Exo70 is the only subunit for which the structure of the almost full-length protein is available so far. As already mentioned, Exo70 features a unique repeated-helical-bundle exocyst organization and it is remarkably elongated [~ 160 Å in length (1 Å = 0.1 nm)] [13,25]. Noticeably, yeast Exo70 can interact with Rho3p-GTP, mediating Exo70's interaction with

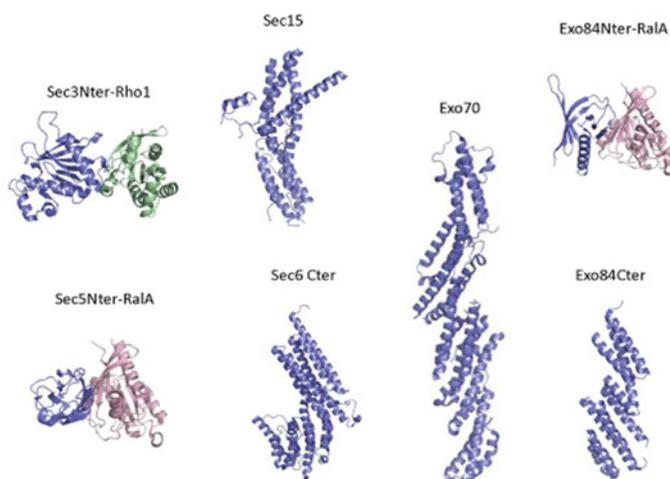


Figure 2 Exocyst subunit structures

The exocyst subunits are in light blue, RalA in light pink and Rho1 in light green. The structures have been represented using PDB files cited in Table 1 and PyMOL (DeLano Scientific; <http://www.pymol.org>). A three-dimensional interactive version of this Figure is available at <http://www.BiochemJ.org/bj/433/0403/bj4330403add.htm>. Cter, C-terminal.

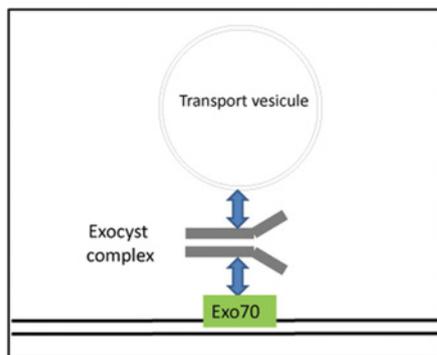


Figure 3 A model of the assembled mammal exocyst complex

A schematic representation of the exocyst complex which would form a 'T' shape. In mammalian cells, Exo70 may act as a scaffold protein for the exocyst's recruitment at specific sites at the PM.

the PM [26,27]. Whereas this interaction is not conserved in mammalian Exo70, a phospholipid-binding motif is present in both yeast and mammalian Exo70 and mediates the recruitment of these proteins to the PM through binding to PIP₂ (phosphatidylinositol 4,5-bisphosphate) [28,29]. In addition, yeast Exo70 has been reported to simultaneously directly interact with three other exocyst subunits (Sec6, Sec8 and Sec10) using distinct binding interfaces [13]. Taken together, these data suggest a key role for Exo70, possibly as a core assembly subunit of the exocyst complex. In addition, as Exo70 stably localizes to the PM, it may act as a landmark for exocyst-mediated tethering events (Figure 3) [28,29]. Tethering represents the first contact between the exocytic vesicle and its target membrane (i.e. the PM), conferring specificity in vesicle-targeting prior to fusion with the acceptor membrane mediated by SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein receptor) proteins (Figure 3) [30,31].

Much less is known regarding the global organization of the exocyst complex. Quick-freeze deep-etch EM (electron microscopy) analysis of the purified mammalian brain exocyst complex has been carried out using either unfixed or glutaraldehyde-fixed preparations to avoid complex dissociation [4]. Unfixed complexes adopted a 'star' shape with four to six extensions, which may indicate some degree of complex dissociation. The structure of the fixed complex displayed a 'T' or 'Y' shape, possibly closer to the native conformation (Figure 3). These data, together with available structural information, allowed Munson and Novick [10] to propose that the elongated rod-like subunits could pack against one another along their long axis (Figure 3). This model requires more direct investigation using antibodies directed against the different subunits (or tagged subunits), in order to refine the architecture of the complex. An additional hypothesis proposed from this model was that the two 'T' arms may form a bridge between the PM and the transport vesicle during the mechanism of tethering (Figure 3) [10]. EM analysis of the complex bound to reconstituted liposomes *in vitro* may possibly help to understand the mechanism of tethering mediated by the complex and identifying core subunits for complex assembly and interaction with membranes.

In the model shown in Figure 3, additional components are required to interface the exocyst complex with the vesicle and PM bilayers. The exocyst complex appears as a central hub for a network of proteins and signalling cascades involved in controlling the docking of exocytic vesicles and cell polarity [32]. On the basis of a number of studies, several members of the Ras

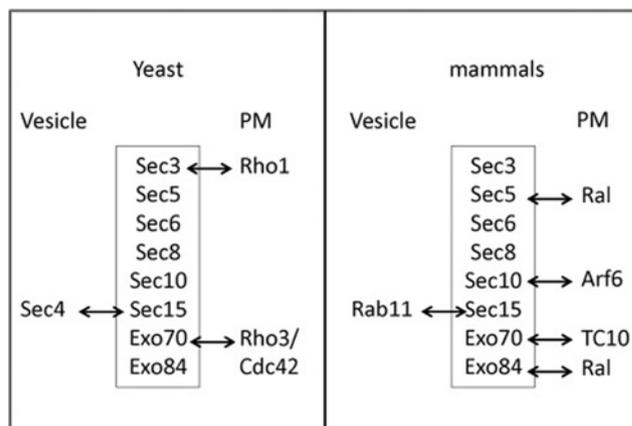


Figure 4 Evolution of the partners of the exocyst complex from yeast to mammals and their localization to the vesicle or PM

superfamily of monomeric GTP-binding proteins have been found to interact with various exocyst subunits in a GTP-dependent manner. These interactions may be instrumental in the process by providing regulated membrane anchors for the exocyst complex on the transport vesicles and with specific regions of the PM (Figure 4). Interestingly, and as noticed earlier, the small GTP-binding protein–exocyst interactome has evolved from yeast to mammals [32]. One illustration of evolutionary remodelling is provided by the mammalian counterparts of Sec3 and Exo70 that apparently lost the binding sites for Rho1/Rho3 and Cdc42 (cell division cycle 42) respectively [33]. In addition, beside Ras-like GTP-binding proteins, a diversity of exocyst partners has been identified thanks to biochemical and yeast two-hybrid approaches [34–36].

In subsequent sections, we focus on a subset of these exocyst-interacting partners involved in the control of motile processes in mammals.

AN EXOCYST COMPLEX NETWORK INVOLVED IN CO-ORDINATING INTRACELLULAR TRAFFICKING AND CELL POLARITY DURING CELL MOTILITY

Cell motility and invasive processes are involved in physiological situations such as embryonic development, immune cell function and wound healing, as well as during severe pathological conditions during tumour dissemination and metastasis. Although motile cells can adopt a diversity of morphologies, they are all highly polarized, reflecting the necessity to establish an antero-posterior axis (also referred to as planar polarity) for efficient movement. For decades, cell motility has been studied *in vitro* using cells moving on two-dimensional substrates (Figures 1B and 1C). Although reductionist, these systems have been extremely powerful and generated a model whereby actin-driven extension of PM protrusions at the leading edge, coupled to integrin-based adhesion of the advancing lamellipodia to the substratum, are counterbalanced by actomyosin contraction and cell retraction of the rear of the cell to allow movement of the cell body [37–39]. Additionally, and coupled to actin- and actomyosin-driven processes, this cycling mechanism of protrusion and retraction is thought to involve a front-to-back flow of membranes in which components such as integrins are endocytosed at the rear of the cell and returned to the leading edge [40].

RalA–exocyst interactions at play during cell migration

The Ral subgroup of small GTP-binding proteins comprises the highly related RalA and RalB proteins, who are involved in a variety of cellular processes such as intracellular trafficking, apoptosis, cytokinesis, cell proliferation, oncogenesis and cell migration [8,41–47]. Sec5 and Exo84 have been identified as partners for Ral proteins, and both subunits were shown to interact with the active GTP-bound form of RalA/B in a mutually exclusive manner on the basis of overlapping binding interfaces [17,18].

Expression of a constitutively activated GTPase-defective form of RalA was found to induce filopodia formation in Swiss 3T3 fibroblasts [48]. Filopodia are actin-based rod-like cell-surface projections of the front edge of motile cells with sensory and exploratory functions, such as sensing filopodia of the tip of growth cones of developing neurons [49]. Previously, filopodia have been shown to probe the surrounding matrix environment and guide cell movement via activated $\beta 1$ integrins [50].

Injection of anti-Sec5 blocking antibodies, which interfered with Sec5 binding to Ral, demonstrated that this interaction was required for the filopodia formation induced in response to several inflammatory cytokines [e.g. TNF α (tumour necrosis factor α) and IL-1 (interleukin-1)] or to the active forms of RalA or Cdc42, acting as master signalling intermediates for these cytokines [48]. Interestingly, the adversary effect of anti-Sec5 antibody is not due to a general secretion-block as Brefeldin A, a general inhibitor of secretion, does not affect RalA-dependent filopodia formation [48]. Knockdown of Sec5 (and other exocyst subunits including Sec8 and Sec10) has been shown to inhibit cell motility and directional migration in a wound-healing assay in NRK (normal rat kidney) fibroblastic cells [8]. Interestingly, the anti-migratory effect of Sec5 siRNA (small interfering RNA) could be phenocopied by knocking down RalB, but not RalA [8]. Along similar lines, RalB was specifically required for the recruitment of the exocyst complex at the leading edge of wound-healing cells [8] (Figure 1B). In addition, in Dunning R3327–5' rat prostate tumour cells the exocyst localizes to the leading edge of motile cells in a Ral-dependent manner, and it co-localizes with focal-adhesion proteins such as paxillin. Interestingly, knockdown of Sec5 or Sec6 interferes with the delivery of post-Golgi transport vesicles containing $\alpha 5$ integrins that mediate cell adhesion to the substratum [42].

Therefore Ral's mediated control of exocyst function at the leading edge may regulate the delivery of filopodial components such as integrins during filopodia formation and hence act directly on cell motility and guidance (Figure 1B). Previously, the Ral-exocyst pathway was also implicated in the formation of newly identified intercellular connecting structures called TNTs (tunnelling nanotubes), which correspond to thin actin-based membranous tethers involved in cell–cell communication [51–53]. Interestingly, these structures, which are morphologically related to filopodia, may rely on similar mechanisms for their formation.

Role of the exocyst complex in positioning of aPKC (atypical protein kinase C) signalling during cell migration

aPKC ζ and aPKC ι are serine/threonine protein kinases with well-known functions in cell polarity, mainly as components of the Par polarity complex [54]. The Par complex is evolutionarily conserved from worms to humans and it regulates cell polarity, including baso–apical polarity of epithelial cells, but also asymmetric cell division, neuronal differentiation and cell migration [55].

aPKCs act by controlling local signalling events in several motility systems. Upon activation of integrins at the leading edge of migrating astrocytes, a Par6–aPKC ζ complex activates a signalling cascade, involving activation of the Rho GTP-binding protein Cdc42, leading to centrosome repositioning in a region facing the leading edge [56]. More recently, it was found that in NRK cells, aPKCs are necessary for wound healing and they physically interact with the exocyst complex via the scaffold protein kibra [9]. These interactions are required for the localization of aPKCs and exocyst components to the leading edge of migrating cells, where they control the local activation of additional protein kinases, ERK (extracellular-signal-regulated kinase) and JNK (c-Jun N-terminal kinase). In turn, ERK and JNK were shown to control the phosphorylation of paxillin, a component of dynamic focal adhesions. These findings support a role for the exocyst complex together with aPKCs and kibra in the delivery of local stimulatory signals to the leading edge of migrating cells [9,57].

A Ral–exocyst axis regulates integrin-dependent trafficking of lipid rafts

Caveolae are 50–100 nm diameter cholesterol- and shingolipid-rich PM invaginations involved in vesicular trafficking and signal transduction events [58]. CAV1 (caveolin-1) is the main structural component of caveolae. Several studies implicate CAV1 in cell motility and adhesion by contributing to integrin and Rac1 signalling [59]. Fibroblasts derived from CAV1-knockout mice are unable to polarize and show defects in directional migration correlated with altered focal-adhesion dynamics [60–62]. This phenotype correlates with an impairment in skin wound healing in CAV1 $^{-/-}$ mice [63]. In addition, loss of integrin-mediated adhesion triggers caveolin-dependent internalization of lipid raft microdomains leading to down-modulation of Rac1-, ERK- and PI3K (phosphoinositide 3-kinase)-dependent pathways [64]. Conversely, re-adhesion of the cells to the extracellular matrix induces the re-insertion of cholesterol- and shingolipid-rich lipid rafts to the PM, restoring anchorage-dependent signalling. Previous studies identified a two-step mechanism for lipid raft exocytosis to the PM after integrin–receptor engagement. These mechanisms, for adhesion-dependent raft exocytosis, are based on the small GTP-binding protein ARF6 (ADP-ribosylation factor 6) and microtubules on the one hand [65], and on RalA and the exocyst complex on the other [43]. Interestingly, active RalA, possibly with the exocyst complex, was able to promote PM localization of lipid rafts in cells in suspension and promoted anchorage-independent growth signalling, potentially linking the exocyst complex to anchorage independence and cancer progression [43].

Interaction of the exocyst complex with actin polymerization machineries

The Arp2/3 complex

Zuo et al. [66] first reported a direct interaction between the Arp2/3 complex and the C-terminal region of Exo70. The Arp2/3 complex functions as an actin nucleator allowing *de novo* assembly of actin filaments [67]. It is involved in a variety of actin-based processes, including lamellipodia extension during cell migration, and it is also implicated in membrane-trafficking events such as endocytosis, in which force generation by actin polymerization may be required for the mechanism of vesicle fission [68]. Loss of function of Exo70 results in defects in actin assembly and the formation of membrane protrusion at the leading edge, both

of which are associated with cell-motility deficiency [66]. These data led to the proposal that in addition to its role in targeted exocytosis at the cell leading edge, the exocyst complex may also regulate lamellipodial actin assembly by interacting with, and recruiting, the Arp2/3 complex at sites of membrane extension [66]. Therefore the exocyst complex appears to be involved in the early stages of polarity establishment through its link with aPKC but also in later stages by interacting with, and possibly regulating, the Arp2/3 complex. Moreover, this association raises issues about a role for actin polymerization during exocytosis. Future studies should reveal how Exo70 can control actin assembly during exocytosis.

IQGAP1 (IQ motif containing GTPase-activating protein 1)

The exocyst complex has been found to interact with the multifunctional scaffolding protein IQGAP1, providing further links with the actin cytoskeleton [69]. IQGAP1 acts downstream of Rho GTP-binding proteins and is involved in actin cytoskeleton assembly, cell adhesion and cell motility [70]. In addition, IQGAP1 is overexpressed in several types of human tumours and is associated with metastasis [71–73]. The exocyst subunits, Sec3 and Sec8, were found to interact with IQGAP1 and this interaction appears to be controlled by the small GTPases Cdc42 and RhoA [69]. Interestingly, in highly invasive breast adenocarcinoma cells, IQGAP1 and Sec8 co-localized at invadopodia [69]. These specific structures of invasive tumour cells correspond to actin-rich PM extensions endowed with the capacity to degrade the underlying extracellular matrix and hence invadopodia are thought to facilitate the dissemination of tumour cells through the body [74] (Figure 1D). IQGAP1, the exocyst complex and Cdc42/RhoA are required for invadopodial proteolysis of the matrix and breast tumour-cell invasion, and loss of their function correlates with impaired delivery of MT1-MMP [membrane type 1 MMP (main matrix metalloproteinase)], the main MMP of invadopodia [69]. Another study also revealed the importance of the interaction between the exocyst and the Arp2/3 complex for invadopodia formation [75]. These data highlight the key role of the exocyst complex in co-ordinating cytoskeletal assembly and exocytosis for invadopodia function and for tumour cell invasion in complex three-dimensional matrix environments (Figure 1D).

THE EXOCYST COMPLEX IS REQUIRED FOR POLARITY ESTABLISHMENT AND NEURITE EXTENSION AND BRANCHING DURING NEURONAL DEVELOPMENT

Neuronal development is characterized by a sequence of events ending with synapse formation allowing the transmission of nerve signals. The differentiation of non-polarized neuroblasts (immature neurons) into neurons, with the formation of axons and dendrites, is called neuritogenesis. This process has been characterized in great detail in hippocampal neurons in culture [76]. Shortly after attachment to the substrate, neuroblasts develop a lamellipodium surrounding the cell body. The extension of neurites begins with the localized formation of ruffles and membrane protrusions at intervals around the cell periphery, which leads to the segmentation of the lamellipodium into discrete processes tipped with a growth cone. As neurites continue to elongate at a slow rate, one minor process begins to grow at a much faster rate and develops a larger growth cone, giving rise to the axon. The other processes will then become dendrites [77,78]. Filopodia in neuronal growth cones are continuously exploring the environment around the neuron and are believed to play a leading role during growth-cone pathfinding and

axon guidance. Neuronal polarization enables the formation of functionally and morphologically distinct axons and dendrites [76,79]. *In vivo*, hippocampal and cortical neurons have their axon or dendrite identity defined at the time of formation of their first neurites. This polarization arises, in part, through the specific intracellular trafficking of lipid and protein components, via vesicle intermediates, to appropriate locations in the cell.

Seminal work has shown that the exocyst complex accumulates at sites of neurite outgrowth and domains of synapse assembly [5]. Later, it was shown that a *Drosophila* mutant deficient for *sec5* died because their neuromuscular junctions failed to expand. In *sec5* neurons cultured *in vitro*, neurite outgrowth failed owing to a defect in neuronal membrane trafficking of newly synthesized proteins, whereas neurotransmitter release was unaffected [80,81]. These studies clearly implicate the exocyst complex in the polarization required for neuron development. In addition, neurite branching is essential for the establishment of a complex neuronal network during development or regeneration. This process generally initiates with the appearance of filopodia. Active Ral leads to the formation of filopodia and its interaction with the exocyst is required for neurite branching [82,83].

The Ral–exocyst axis is also involved in neuronal polarization through spatial and temporal regulation of polarity proteins such as aPKC or Par3. Interaction between Par3, aPKC and the exocyst complex increases upon neuronal polarization, leading to the localization of Par3 at restricted sites of the PM in a RalA-regulated manner [82,83]. Thus, as in migrating cells, Ral interacting with the exocyst complex is involved in signal delivery to sites of membrane extension.

More recently, other studies showed that two mutually exclusive modes of neuronal development may co-exist depending on a co-ordination between the actin cytoskeleton and membrane trafficking [77,84,85]. One mechanism is based on Ena/VASP (vasodilator-stimulated phosphoprotein)-regulated actin assembly in concert with VAMP (vesicle-associated membrane protein) 2 [84], whereas a second mechanism relies on Arp2/3 complex-mediated actin polymerization and VAMP7-dependent exocytosis [84]. VAMP2 and VAMP7 belong to the SNARE protein family and mediate fusion of the trafficking vesicle to acceptor compartment, here the PM. VAMP7-mediated exocytosis requires the exocyst complex [30]. In neurons lacking Ena/VASP proteins, overexpression of VAMP7 or Exo70 can rescue neuritogenesis. That study elucidates a crucial role for the exocyst complex in a specific pathway of exocytosis mediated by the SNARE protein VAMP7, during neuronal development.

CONCLUSIONS

The exocyst complex appears to mediate tethering events occurring at the PM. Specificity for the PM is dictated by the many interactions of several exocyst complex subunits with various small GTP-binding proteins and specific lipids [30,31]. In the case of Exo70, localization is strictly dependent on a direct interaction with PIP₂. This lipid, which localizes to the cytoplasmic leaflet of the PM, is implicated as a cytoskeletal anchor and it serves as attachment site for various cellular factors and activities that regulate actin assembly, which are crucial during cell migration and integrin-mediated adhesion [86,87]. By interacting with PIP₂, the exocyst complex is thus at the crossroad of several pathways co-ordinating actin cytoskeleton assembly, exocytosis and signalling during cell motility. The role of PIP₂ and other lipids on the regulation of tethering mediated by the exocyst complex remains poorly understood and should benefit from future studies using *in vitro* reconstitution with synthetic

liposomes. Additionally, biochemical and structural approaches have led to the identification of a novel protein fold which is a signature for exocyst subunit components but, interestingly enough, is also shared by other tethering factors. Further effort is required to unravel the general organization of the complex, its mechanism of assembly and the role of core subunits such as Exo70 in this process. It is possible that Exo70 comprises a landmark for exocyst assembly, either constitutively or in a regulated manner through the action of PIP₂ or small GTP-binding proteins. These issues will have to be carefully addressed in the future.

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