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Membrane targeting of the yeast exocyst complex

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ABSTRACT

The exocytosis is a process of fusion of secretory vesicles with plasma membrane, which plays a prominent role in many crucial cellular processes, e.g. secretion of neurotransmitters, cytokinesis or yeast budding. Prior to the SNARE-mediated fusion, the initial contact of secretory vesicle with the target membrane is mediated by an evolutionary conserved vesicle tethering protein complex, the exocyst. In all eukaryotic cells, the exocyst is composed of eight subunits — Sec5, Sec6, Sec8, Sec10, Sec15, Exo84 and two membrane-targeting landmark subunits Sec3 and Exo70, which have been described to directly interact with phosphatidylinositol (4,5)-bisphosphate (PIP_2) of the plasma membrane. In this work, we utilized coarse-grained molecular dynamics simulations to elucidate structural details of the interaction of yeast Sec3p and Exo70p with lipid bilayers containing PIP_2. We found that PIP_2 is coordinated by the positively charged pocket of N-terminal part of Sec3p, which folds into unique Pleckstrin homology domain. Conversely, Exo70p interacts with the lipid bilayer by several binding sites distributed along the structure of this exocyst subunit. Moreover, we observed that the interaction of Exo70p with the membrane causes clustering of PIP_2 in the adjacent leaflet. We further revealed that PIP_2 is required for the correct positioning of small GTPase Rho1p, a direct Sec3p interactor, prior to the formation of the functional Rho1p-exocyst-membrane assembly. Our results show the critical importance of the plasma membrane pool of PIP_2 for the exocyst function and suggest that specific interaction with acidic phospholipids represents an ancestral mechanism for the exocyst regulation.

1. Introduction

All eukaryotic cells display some aspects of polar organization, which, in essence, is achieved by the asymmetric localization of intracellular structures and components. This phenomenon has been described to play a critical role in the development of neuronal cells, in the growth of fungal hyphae or plant pollen tubes and root hairs [1–3]. The cell polarity is mediated by the asymmetric distribution of proteins and lipids in the specialized domains in the plasma membrane (PM). The polar domains are formed by properly balanced exocytosis, endocytosis and two-dimensional lateral mobility within PM [4]. Exocytosis is a process of the fusion of secretory vesicles with PM. Secretory vesicles, that bud from the trans-Golgi network or recycling endosome, are transported along cytoskeletal networks to their destination, where they are tethered and finally fused with PM. The tethering step, described as the initial contact of the secretory vesicle with the target PM prior to the SNARE-catalyzed membrane fusion [5], is in many cases mediated by the evolutionary conserved multiprotein complex, the exocyst. All subunits of the exocyst were discovered in the budding yeast by genetic screens for mutants in secretion [6] and the budding yeast has been an invaluable model in identifying basic principles of the exocyst functions. In addition to its mechanistic role in tethering, the exocyst also determines the region of PM, where exocytosis takes place. The exocyst is composed of eight subunits, Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84. The whole exocyst complex has the molecular weight of approximately 750 kDa with subunits ranging in size from 70 to 150 kDa [7]. The exocyst is a member of the structurally and functionally related Complex Associated with Tethering Containing Helical Rods (CATCHR) family, which also contains COG, Dsl and GARP complexes, that tether vesicles at the Golgi, ER and endosomes, respectively [8]. Several recent studies have provided valuable insights into the structural organization of the exocyst complex [9]. So far, partial crystal structures of five exocyst components have been obtained. These include near full-length of yeast Exo70 (Exo70p, Fig. 1), the C-terminal part of Exo84p and Sec6p, and the N-terminal part of Sec3p [10–14]. In addition, the crystal structures of the near full-length mouse Exo70, the C-terminal part of Drosophila Sec15 and the Ral-binding domain of rat Exo84 were solved [15]. Yeast Exo70p (amino acid residues 67–623) folds into an elongated rod that is 160 Å long and 30–35 Å wide, it comprises nineteen α-helices and

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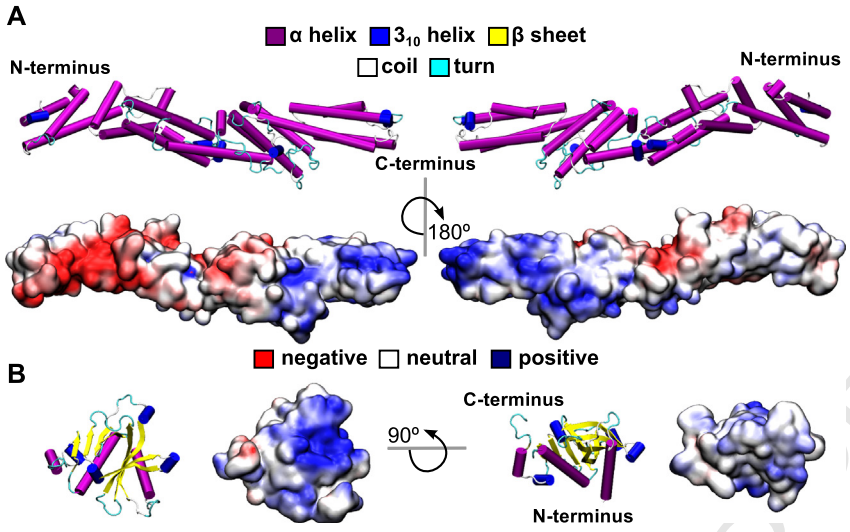

Fig. 1. Both Exo70p and Sec3p-N contain positively charged regions. A. The structure of Exo70p (PDB ID: 2PFV) and electrostatic potential mapped onto the solvent accessible surface calculated using the APBS program [70]. B. The structure of Sec3p-N (PDB ID: 3A58) and electrostatic potential mapped onto the solvent accessible surface. Electrostatic potential spans from −5 (red) to +5 (blue) kbT/e_c.

the whole structure could be divided into four domains (Fig. 1A). Despite the low sequence identity, all experimentally solved structures of exocyst subunits share the rod-like structural core composed of two or more consecutively packed helical bundles, with each bundle consisting of three to five α-helices linked by loops [16]. Analysis of the electrostatic potential mapped on the Exo70p surface revealed distinct polarity of the Exo70p structure [10,11] (Fig. 1A), where its N-terminal part is strongly negatively charged and the C-terminal part is positively charged. Interestingly, the prediction of the secondary structure suggests that the remaining unsolved exocyst subunits may be formed by similar helical folds [17]. In contrast to the rod-like structures described above, the crystal structure of N-terminal domain of Sec3p (Sec3p-N) revealed that this part folds into the Pleckstrin homology (PH)-like domain [13,14] (Fig. 1B). Sec3p-N (amino acid residues 76–260) is composed of three α-helices (α1–α3) and eight β-strands (β1–β8). The β1–β7 strands form an antiparallel β sheet resulting in the orthogonal sandwich and the abutting α2-helix typical for the PH-fold [14]. Examination of the electrostatic surface of Sec3p-N showed a positively charged pocket formed by three clusters of basic residues: i) R137 and R157, ii) R137, K135 and K155, and iii) R168 and K194 [14] (Fig. 1B).

A crucial aspect of the exocyst function is its tightly controlled interaction with PM. In the budding yeast cell, all exocyst subunits are polarized to the growing end of the daughter cell (bud tip), but the mechanisms of their targeting differ [16]. Although several alternative models have been proposed, Exo70p and Sec3p are generally believed to mediate the contact with the target PM region and thus ensure the specific localization of the whole complex. In most of the current models the remaining exocyst subunits are believed to be delivered to the exocytic sites with secretory vesicles on actin cables [18], but recent data indicate that in different organisms, dynamics of exocyst assembly and targeting may vary significantly [19,20]. A couple of recent reports showed that Sec3p and Exo70p interact with phosphatidylinositol (4,5)-bisphosphate (PIP_2), which is present predominantly in the inner leaflet of PM [21]. Sec3p binds PIP_2 via its N-terminal PH-like domain and basic residues located at the C-terminal part are important for Exo70p interaction with PIP_2 [13,14,21,22]. The disruption of the interaction of PIP_2 with both Sec3p and Exo70p inhibits PM-association of the exocyst and results in severe growth defects [21]. During the last 15 years, PIP_2 has emerged as an important second messenger especially in the regulation of cytoskeletal and membrane dynamics [23,24]. The distinctive features of PIP_2 are its bulky acidic headgroup, with the net charge ranging from −3 to −5 under physiological pH and an inverted conical overall shape that promotes positive curvature of membranes. PIP_2 functions by recruiting effector proteins to membranes in a spatio-temporally specific manner and/or it affects the biophysical properties of membranes.

To fully understand the exocyst function, it is important to explore the interaction of membrane-associating subunits of the exocyst and PIP_2 in the context of lipid bilayer. Molecular dynamics (MD) simulations have been shown to play an invaluable role in the molecular-level description of dynamic interaction of membrane proteins with phospholipids, which could be hardly obtained by experimental approaches [25,26]. In this study, we utilize coarse-grained MD simulations to reveal details of the specific Exo70p and Sec3p interaction with PIP_2. We also address simultaneous binding of Sec3p to PIP_2 and small GTPase Rho1p. Our results show an importance of the mutual interplay between PIP_2 molecules and proteins involved in exocytosis.

2. Methods

2.1. Structures used in the simulations

Before converting Exo70p structure (PDB ID: 2PFV, resolution of 2.10 Å, residues 67–623) [27] to the CG representation, the missing loop ILE224-PRO231 in the crystal structure was added using the program MODELLER 9.14 [28]. The structure of Sec3p-N was solved together with Rho1p (PDB ID: 3A58, resolution of 2.60 Å) [14] and both structures were used as a starting point for subsequent simulations. To convert exocyst subunits to the MARTINI v2.1 CG representation, we utilized the martinize.py script and we used the ELNEDYN representation with r_c = 0.9 nm and f_c = 500 kJ mol^{-1} nm^{-2} [29–31]. In the crystal structure of Sec3p-N-Rho1p, the C-terminal part of Rho1p called hypervariable region (HVR, amino acid residues 186 to 209) is missing, we therefore modeled this part as a random coil using the MODELLER 9.14 program. The ELNEDYN representation with r_c = 0.9 nm and f_c = 500 kJ mol^{-1} nm^{-2} was used to describe core of Rho1p and HVR was kept flexible. The C-terminal cysteine residue 207 of Rho1p was geranylgeranylated. The model of the geranylgeranyl tail consisted of a linear sequence of four C3 MARTINI beads, similarly to the published model of farnesyl moiety [32], all connected via harmonic bonds with r_0 = 0.49 nm and f_c = 8000 kJ mol^{-1} nm^{-2}. The angle bending was restricted by harmonic potentials with a = 140 equilibrium angle and f_c = 200 kJ mol^{-1}. The first C3 bead was connected to the cysteine side chain bead using a harmonic bond with r_0 = 0.39 nm and f_c = 5000 kJ mol^{-1} nm^{-2}.

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