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Ca^{2+} Signaling Proteins and Mammalian Sec6/8 Complex

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Polarized expression of signaling complexes requires mechanisms for delivery, assembly and retention of the proteins in the microdomains. Little or no information is available as to the nature of these mechanisms in non-excitable cells. It is possible that the vesicles/protein delivery system known as the exocyst or Sec6/8 complex participate in targeting the Ca^{2+} signaling complex to the microdomain. The data suggested by our work demonstrate that the sec6/8 complex mediated delivery of pre-assembled Ca^{2+} signaling complexes to the apical pole region most proximal to the tight junctions. A novel function of the Sec6/8 complex is that the Sec6/8 complex has multiple roles in secretory cells including governing the polarized expression of Ca^{2+} signaling complexes and regulation of their activity.

Keywords: Sec6/8 Complex, Ca^{2+} Signaling Proteins, Polarity, exocrine cells

An increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) drives gene expression, initiates proliferation, stimulates fluid and electrolyte secretion, triggers fertilization, contracts muscle, and stimulates synaptic transmission (Berridge, 1993; Muallem and Wilkie, 1999). Like other cells, exocrine cells respond to a series of hormones and neurotransmitters that activate G-protein-coupled receptors (GPCRs). GPCR-dependent Ca^{2+} signaling complexes are composed of two components, a biochemical component and a biophysical component. A biochemical component consists of GPCRs, heterotrimeric G proteins and phospholipase C β (PLC β). Conformational change of Gaq·GTP stimulates PLC β to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP_2) and release IP_3 to the cytosol. A biophysical component consists of two Ca^{2+} channels, IP_3 receptor channels (IP_3R) and Ca^{2+} released Ca^{2+} activated channels (Icrac), and two Ca^{2+} pumps, plasma membrane Ca^{2+}-ATPase (PMCA) and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). IP_3 activates the IP_3R in the endoplasmic reticulum (ER) to release Ca^{2+} from the ER and increase the $[Ca^{2+}]_i$. Ca^{2+} release is followed by activation of Icrac, the Ca^{2+} influx channels in the plasma membrane (PM) (Putney and McKay, 1999). Activation of the IP_3R and Icrac occurs in the rapid increase in $[Ca^{2+}]_i$ and in the next phase, the PMCA and the SERCA pumps remove Ca^{2+} from the cytosol to reduce $[Ca^{2+}]_i$ back towards resting level (Muallem, 1992). At continuous stimulation, $[Ca^{2+}]_i$ stabilizes at a level determined by the relative activities of the biochemical and biophysical components, Ca^{2+} channels and pumps (Berridge, 1993; Muallem, 1992; Putney and McKay, 1999). At physiological stimulus intensity, the sequence of events leading to the transient change in $[Ca^{2+}]_i$ is periodically repeated, giving rise to $[Ca^{2+}]_i$ oscillations (Berridge, 1993). Ca^{2+} signaling in secretory cells is highly polarized (Muallem and Wilkie, 1999). Agonist stimulation triggers Ca^{2+} signals in the form of Ca^{2+} waves that are initiated in the luminal pole and propagate along the cell periphery and the lateral membrane to the basal pole (Kasai *et al.*, 1993). This phenomenon was later confirmed in pancreatic acinar cells (Thorn *et al.*, 1993; Nathanson *et al.*, 1994; Xu *et al.*, 1996) and extended to other exocrine cells (Lee *et al.*, 1997a; Yamamoto-Hino, *et al.*, 1998). Subsequent studies showed that expression of high levels of all IP_3R isoforms at the apical pole accounts for the initiation of $[Ca^{2+}]_i$ waves at this site (Lee *et al.*, 1997a; Yule *et al.*, 1997). In polarized exocrine cells such as pancreatic and salivary gland acini, apical pole showed higher sensitivity to Ca^{2+} release by IP_3 than other regions of the cell, including the basal pole (Kasai *et al.*, 1993; Fogarty *et al.*, 2000). Likewise, plasma

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membrane Ca^{2+} pumps (PMCA) (Lee *et al.*, 1997b; Zhao *et al.*, 2001) is expressed at high levels in the luminal pole. The sarco/endoplasmic reticulum Ca^{2+} Pumps (SERCA) are expressed in a cell and region specific manner with high levels along the lateral and subapical region (Lee *et al.*, 1997b). The same lateral-subapical region is enriched with several G-protein coupled receptors (Rios *et al.*, 1999). Recently we reported that GPCR M3 and CCK receptors are expressed at high levels adjacent to the tight junctions at the luminal pole (Shin *et al.*, 2001). Accordingly, Ca^{2+} release from the apical pole is the most sensitive to agonist stimulation and to activation by IP_3 (Kasai *et al.*, 1993; Thorn *et al.*, 1993).

Evidence accumulated over the last few years, particularly in polarized cells, begun to answer the question of how the polarized localization of signaling complexes and polarized exocytosis are achieved. The mechanism by which the exocytotic proteins promote vesicle targeting is unclear. However, the exocyst or Sec6/8 complex may determine signaling polarity since it is proposed to play a central role in the establishment of cell polarity from yeast to mammals (Finger and Novick, 1998; Hsu, *et al.*, 1999). Proteins of the exocyst were first identified in yeast as proteins involved in exocytosis (Bowser *et al.*, 1992). Later, the proteins were found to form a multi-subunit complex termed the exocyst. Genetic and biochemical analysis showed the exocyst to contain 8 subunits, Sec3p/5p/6p/8p/10p/15p/Exo70p/Exo84p (TerBush *et al.*, 1996, Guo *et al.*, 1999) with molecular weights ranging from 70 to 144 kDa. During cytokinesis, the exocyst re-concentrates in a ring-like structure at the neck of mother cell and the bud. Bud tip and mother-daughter neck represent sites of directed membrane growth that is coordinated with the cell cycle (TerBush and Novick, 1995; Finger *et al.*, 1998; Finger and Novick, 1998). The Sec3 subunit serves as a landmark for vesicles delivery since its localization is independent of the secretory pathway and the actin cytoskeleton (Finger *et al.*, 1998). Furthermore, the localization of Sec3 seems to be independently of the other subunits of the exocyst (Finger *et al*, 1998). Although the function of the other subunits is not known at present, deletion of individual subunits and/or expression of dominant negatives result in accumulation of secretory vesicles in the cytoplasm and non-polarized exocytosis and cell growth (Finger and Novick, 1998). Hence, in yeast the exocyst mediates the polarized delivery of secretory vesicles to regions of active exocytosis through a series of protein-protein interactions among the exocyst proteins(Finger and Novick, 1998).

The exocyst has been refered to as Sec6/8 complex because most of the work in mammalian system has been performed using antibodies directed against Sec6 and Sec8 subunits. The mammalian Sec6/8 complex was originally purified from rat brain and shown to comprise of 8 subunits, analogues to those of the yeast exocyst (Hsu *et al.*, 1996). Accumulating evidence indicates that the Sec6/8 complex is required for post-Golgi vesicle trafficking (Guo *et al*, 2000). The analogue of yeast Sec3, Human Sec3 (hSec3), shares 17% sequence identity with yeast Sec3p, expressed in almost all tissues (Matern *et al.*, 2001). Furthermore, strong evidence suggests that the mammalian Sec6/8 complex participates in polarized delivery of vesicles. In the epithelial cell line MDCK cells, the Sec6/8 complex regulates vesicle targeting to the basolateral, but not to the apical membrane (Grindstaff *et al.*, 1998). In developing cultured hippocampal neurons, the Sec6/8 complex localizes to regions of cell growth, including axonal and dendritic growth cones as well as sites of synaptic formation along axons (Hazuka *et al.*, 1999).

Functionally, localization of the Sec6/8 complex is dynamically regulated. In yeast, the exocyst localizes to the bud tip until the bud is about one-half the size of the mother. Then it is disassembled and reformed at the site of cell division (Finger and Novick, 1998). In non-polarized MDCK cells, the complex is soluble in the cytosol. Upon formation of cell-cell contacts, it is found in cell-cell interacting domains on initiation of calcium-dependent cell-cell adhesion. As cell polarity develops, the localization of the complex becomes restricted to the apical junctional complex, which includes adherens junctions and tight junctions. Therefore, in polarized monolayers, the Sec6/8 complex co-localizes with ZO1 at or near tight junctions (Grindstaff *et al.*, 1998). After establishment of stable synapses, the Sec6/8 complex is no longer enriched in synapses (Hazuka *et al.*, 1999). In differentiated PC12 cells the complex is found in the cell body, in the extending neurite, and at the growth cone, whereas it shows a perinuclear localization in undifferentiated PC12 cells (Matern *et al*, 2001). In yeast, this dynamic translocation of the Sec6/8 complex is maybe mediated by the actin cytoskeleton. Disruption of the actin cytoskeleton prevents the polarized localization of Sec8 (Ayscough *et al.*, 1997; Finger *et al.*, 1998). However, in undifferentiated PC12 cells, Sec6/8 complex is associated with microtuble organizing center, promoting neurite outgrowth, not actin cytoskeleton (Vega and Hsu, 2001). In other hands, the mammalian Sec6/8 complex has been suggested to interact with the cytoskeletal septins (Hsu *et al.*, 1998).

Ca^{2+} signaling proteins in polarized cells are mainly localized at tight junction, suggesting Ca^{2+} signaling proteins complex may exist in a specific cellular microdomain. Recent data showed that the localization of IP_3R is apical (Lee *et al.*, 1997a, Shin *et al.*, 2001) and IP_3 or ryanodine receptors can interact with TRP1 (Singh *et al.*, 2001) and 3 (Kiselyov *et al*, 1999, 2000) demonstrating molecular interactions between plasma Ca^{2+} channel and ER-resident Ca^{2+} channel. Therefore, it is possible that unknown scaffolding proteins are responsible for maintaining and delivering expression of polarized Ca^{2+} signaling proteins. The mammalian Sec6/8 complex is ubiquitously expressed (Hsu *et al.*, 1999), but its function has been examined only in MDCK and neuronal cells. Nothing is known about the

localization and function of the Sec6/8 complex in fully differentiated cells. We examined the localization and possible roles of the Sec6/8 complex in modulating Ca^{2+} signaling in pancreatic acinar cells. Immunolocalization by our group showed that part of the Sec6/8 complex form a basket around secretory granules. Another part of the complex associates with Ca^{2+} signaling complexes to regulate their activity. The later was confirmed by the fact that Abs against Sec8 and Sec6 abolishes calcium waves induced by carbachol, but not calcium waves induced by a non-metabolizable IP_3 (Shin *et al.*, 2000). However, our data did not demonstrate the exact localization of Sec6/8 complex in microdomain except the possibility of localization of perinuclear and plasma membrane lesion. Vega and Hsu (2001) found the similar localization of the Sec6/8 complex in fully differentiated cells being peri-nuclear. Recently, Nelson group demonstrated that the Sec6/8 complex localize on exact trans-Golgi network and plasma membrane in normal rat kidney cell, suggesting steady-state distribution of Sec6/8 complex depends on continuous exocytic vesicle trafficking (Yeaman *et al.*, 2001). It is not clear that Sec6/8 complex is associated with Golgi network in fully differentiated cells.

The central finding of our work in Fig. 1 is that Sec6/8 complex can be co-immunoprecipitated with Ca^{2+} signaling proteins and disruption of this interaction disrupts Ca^{2+} signaling (Shin *et al.*, 2000). The Sec6/8 complex proteins are predicted to be soluble proteins, yet they mostly associate with cellular membranes in neurons (Hazuka *et al.*, 1999), differentiated MDCK cells (Grindstaff *et al.*, 1998) and pancreatic acini. Our work suggests that the actin cytoskeleton plays a critical role in this association. In yeast, disruption of the actin cytoskeleton by lantrunculin A inhibited the polarized accumulation of exocyst proteins (Ayscough *et al.*, 1997; Finger *et al.*, 1998). In pancreatic acini, dissociation of the actin cytoskeleton in intact cells disrupted the localization of the Sec6/8 complex and resulted in translocation of about 50% of the complexes from the membrane to the cytosol. Notably, dissociation of actin filaments also caused the dissociation between the Sec6/8 complex and Ca^{2+} signaling proteins. This finding implies that the Sec6/8 and Ca^{2+} signaling complexes do not interact directly, but rather the Sec6/8 complex is recruited to Ca^{2+} signaling complexes with the aid of the actin cytoskeleton. This may occur while the Sec6/8 complex delivers the Ca^{2+} signaling proteins from the ER and the Golgi to their destination in plasma membrane microdomains. Our results suggest that the Sec6/8 complex has several, probably related functions in polarized epithelial cells, including governing localization and regulating the function of the Ca^{2+} signaling machinery.

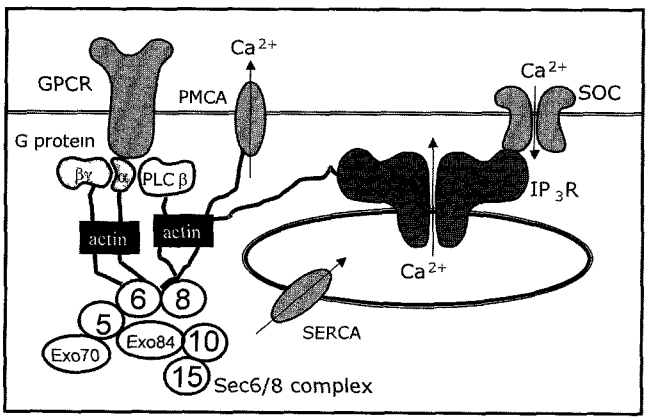

Fig. 1. Ca^{2+} signaling proteins and mammalian Sec6/8 complex. Sec6/8 complex is composed of Sec5p/6p/8p/10p/15p/Exo70p/Exo84p. Sec6/8 complex mainly co-localizes with Ca^{2+} signaling proteins and have a protein-protein interaction with Ca^{2+} signaling proteins such as IP_3R, $G\beta\gamma$, $G\alpha$, and PMCA with the aid of actin cytoskeleton near the plasma membrane. Disruption of actin cytoskeleton abolishes agonist-induced Ca^{2+} wave and localization of Sec6/8 complex, indicating multiple role of Sec6/8 complex including governing localization and regulating the function of the Ca^{2+} signaling machinery. IP_3R, inostol 1,4,5-trisphosphate receptor; PMCA, plasma membrane Ca^{2+} ATPase.

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