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Exocyst Sec10 protects renal tubule cells from injury by EGFR/MAPK activation and effects on endocytosis

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Fogelgren B, Zuo X, Buonato JM, Vasilyev A, Baek JI, Choi SY, Chacon-Heszele MF, Palmyre A, Polgar N, Drummond I, Park KM, Lazzara MJ, Lipschutz JH. Exocyst Sec10 protects renal tubule cells from injury by EGFR/MAPK activation and effects on endocytosis. Am J Physiol Renal Physiol 307: F1334–F1341, 2014.—Acute kidney injury is common and has a high mortality rate, and no effective treatment exists other than supportive care. Using cell culture models, we previously demonstrated that exocyst Sec10 overexpression reduced damage to renal tubule cells and sped recovery and that the protective effect was mediated by higher basal levels of mitogen-activated protein kinase (MAPK) signaling. The exocyst, a highly-conserved eight-protein complex, is known for regulating protein trafficking. Here we show that the exocyst biochemically interacts with the epidermal growth factor receptor (EGFR), which is upstream of MAPK, and Sec10-overexpressing cells express greater levels of phosphorylated (active) ERK, the final step in the MAPK pathway, in response to EGF stimulation. EGFR endocytosis, which has been linked to activation of the MAPK pathway, increases in Sec10-overexpressing cells, and gefitinib, a specific EGFR inhibitor, and Dynasore, a dynamin inhibitor, both reduce EGFR endocytosis. In turn, inhibition of the MAPK pathway reduces ligand-mediated EGFR endocytosis, suggesting a potential feedback of elevated ERK activity on EGFR endocytosis. Gefitinib also decreases MAPK signaling in Sec10-overexpressing cells to levels seen in control cells and, demonstrating a causal role for EGFR, reverses the protective effect of Sec10 overexpression following cell injury in vitro. Finally, using an in vivo zebrafish model of acute kidney injury, morpholino-induced knockdown of sec10 increases renal tubule cell susceptibility to injury. Taken together, these results suggest that the exocyst, acting through EGFR, endocytosis, and the MAPK pathway is a candidate therapeutic target for acute kidney injury.

AKI; EGFR; exocyst; MAPK

ACUTE KIDNEY INJURY IS A SIGNIFICANT and increasing problem that occurs in 1–7% of hospitalizations and up to 25% of intensive care unit (ICU) admissions. Mortality rates in affected patients, especially in ICUs, can be as high as 50–70% (1, 4, 8, 26, 42, 47). Acute kidney injury is also a significant contributor to the progression of chronic kidney disease (45, 49). Unfortunately, there are still no effective treatments for acute kidney injury other than supportive care. Acute kidney injury most often results from a decrease in effective blood flow to the kidney, such as during an ischemia and reperfusion injury. In this case, reactive oxygen species are generated and renal tubular epithelial cells are severely damaged. The tubular cells lose their ability to form a tight epithelial barrier or maintain polarity, and substantial numbers undergo cell death by apoptosis and/or necrosis. Dead cells are sloughed into the lumen, while dedifferentiation and spreading of remaining viable cells attempt to cover the denuded areas. These remaining tubular cells struggle to proliferate, re differentiate, and reestablish polarity and a tight epithelial barrier with normal physiological activity (41). To find novel therapeutic targets for acute kidney injury, it seems critical to identify factors that either mediate renal tubule cell damage or stimulate epithelial recovery following injury.

Protein trafficking is essential for such crucial cell functions as delivery of membrane and secreted proteins, establishment and maintenance of polarity, and complex morphogenesis such as tubulogenesis. An essential step in this process involves targeting and docking secretory vesicles carrying polarized proteins, which is mediated by the exocyst complex (24). This is followed by fusion of these vesicles with the plasma membrane and release of the vesicular protein cargo (37). The 750-kDa exocyst complex is comprised of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (40) and is highly conserved from yeast to humans (16, 30). Sec10 and Sec15 are the most vesicle proximal exocyst components and act as a bridge between Rab GTPases found on the surface of the secretory vesicles and the rest of the exocyst complex in contact with the plasma membrane (12). We and others previously showed that the exocyst traffics primarily basolateral membrane proteins in polarized epithelial cells (10, 22).
The Sec10 subunit is thought to be a central component of the exocyst complex, and modifying levels of Sec10 expression has been shown to result in parallel changes in overall exocyst activity. In yeast, overexpression of a Sec10 NH2-terminal dominant negative displaced the full-length Sec10 from the exocyst complex and resulted in a block of exocytosis and accumulation of secretory vesicles (36). In mammals, overexpression of the NH2-terminal Sec10 fragment acted as a dominant negative and inhibited neurite outgrowth (44). We previously showed that shRNA-mediated knockdown of Sec10 caused protein degradation of other exocyst subunits and decreased exocyst trafficking activity in mammalian epithelial cells (52). Conversely, overexpression of only the Sec10 subunit led to increases in exocyst-mediated trafficking to basolateral surfaces (22) and primary cilia (52). Sec10 overexpression also induced phenotypic changes such as increased tubulogenesis in Madin-Darby canine kidney (MDCK) cell cysts (22).

A well-described in vitro model of the oxidative stress that renal tubule cells incur during acute kidney injury is a brief treatment with H2O2 (39). Following H2O2 treatments, the degree of injury and the time it takes for the injured renal tubule cells to recover their barrier integrity can be measured using transepithelial resistance (TER) as a marker (23, 28, 34). We recently found that stable overexpression of Sec10 in MDCK cells significantly increased phosphorylated (active) ERK, decreased the degree of H2O2 injury, and increased the rate of epithelial barrier recovery following injury. If the MEK inhibitor U0126 was added to prevent ERK phosphorylation, recovery after H2O2 injury was decreased to the same rate as control cells. These data indicated that the faster recovery measured in exocyst-enhanced epithelial cells is mediated through the MAPK pathway (34).

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases. Binding of EGF (and other ligands such as transforming growth factor-a) promotes EGFR homo- and heterodimer formation and the autophosphorylation of cytoplasmic regulatory tyrosines that connect the receptor to downstream signaling pathways. Phosphorylated EGFR receptors are internalized primarily via a clathrin-dependent pathway and shuttled to sorting endosomes (20). At this point, phosphorylated EGFR is either sent on to the cell surface (48). EGF binding of EGFR is one of several upstream stimuli that activate the classic MAPK phosphorylation cascade of Raf-MEK-ERK (2).

EGFR gene expression, abundance of EGFR ligands, and EGFR activation have all been shown to increase in a variety of models of experimental acute kidney injury, including ischemia and reperfusion injury (14, 15, 21, 38, 50). Administration of exogenous EGFR ligands has also been shown to accelerate recovery from ischemic acute kidney injury (17, 29). Recently, Chen et al. (3) showed that out of 39 different phosphorylated mouse receptor tyrosine kinases, phospho-EGFR was the most markedly upregulated in response to renal ischemia and reperfusion injury. They went on to show that both administration of erlotinib (a specific EGFR inhibitor) and targeted deletion of the EGFR gene in the renal proximal tubule resulted in markedly delayed structural and functional recovery following ischemia and reperfusion injury (3).

Here we show that the mechanism by which Sec10 overexpression increases ERK phosphorylation and recovery from oxidative injury in renal tubular MDCK cells is EGFR, MAPK, and endocytosis dependent. Furthermore, knockdown of sec10 in vivo in zebrafish results in greater kidney tubule cell damage following photoablation injury.

METHODS

Cell culture and reagents. All MDCK cell lines used were derived from low passage type II MDCK cells that were obtained from Dr. K. Mostov (University of California, San Francisco, CA) and were originally cloned by Dr. D. Louvard (European Molecular Biology Laboratory, Heidelberg, Germany). Human Sec10 cDNA with a myc epitope tag added to the COOH terminus was cloned into the pcDNA3 mammalian expression vector, where expression is driven by a CMV promoter (11). This plasmid was stably transfected into type II MDCK cells, and monoclonal lines were selected that stably overexpress Sec10, as described in Lipschutz et al. (22). Stable monoclonal MDCK cell lines with shRNA-mediated Sec10 knockdown were previously generated and characterized by our laboratory (52). For Sec10-GST pull-downs, full-length human Sec10 cDNA was cloned in frame into the plasmid pGEX-4T-1 (Amersham Biosciences, Piscataway, NJ) and transformed into the DE3 strain of Escherichia coli (Stratagene, La Jolla, CA). GST fusion protein expression was induced by adding isopropyl-1-thio-β-D-galactopyranoside to growing cultures and shaking for an additional 3 h at 37°C. Recombinant proteins were purified with glutathione-Sepharose (Amersham Biosciences) following bacterial cell lysis. For pull-down experiments, lysates from wild-type MDCK cells were incubated for 1 h with Sec10-GST, or GST only, bound to glutathione-Sepharose. Pull-downs were washed extensively and resuspended in Laemmli buffer and boiled. Equal amounts were electrophoresed on SDS-PAGE and analysed with Western blotting.

Western blot analysis. For pull-downs from MDCK cell lysates, immunoblotting was performed with standard methods as previously described (52). Briefly, proteins separated by SDS-PAGE were transferred to either nitrocellulose or PVDF membranes using a semidry transfer system (Bio-Rad). After being blocked with 5% milk, the membranes were incubated with primary antibodies overnight at 4°C. After being washed, the membrane was then incubated with secondary antibodies either labeled with horseradish peroxidase for chemiluminescent detection or with fluorescence (IRDye; LI-COR Biosciences). The LI-COR Odyssey Imager (LI-COR Biosciences) was used for fluorescence detection of proteins, and the LI-COR Image Studio Lite software was used to quantify band intensities. The primary antibodies used in this study were mouse monoclonal anti-GAPDH (G8795; Sigma, St. Louis, MO), anti-phospho-EGFR (Tyr1068, cat. no. 3777), anti-total-ERK (cat. no. 4267), anti-phospho-ERK (Thr202/Tyr204, cat. no. 4370), anti-total-ERK (cat. no. 3189) each from Cell Signaling Technology (Beverly, MA), and a rabbit polyclonal anti-Sec10 that we generated and previously characterized (52).

Statistical analysis of Western blot band intensities was performed with Graphpad Prism software, and P values were calculated using either Student’s t-test (for two-group comparisons) or one-way ANOVA analysis followed by post hoc Tukey multiple comparisons (for multiple group comparisons).

EGF binding and internalization assay. Recombinant human EGF (Peprotech, Rocky Hill, NJ) was labeled with 125I (PerkinElmer, Waltham, MA), and the activity of labeled EGF was determined using a phosphotungstic acid precipitation assay. Rate constants of EGF internalization (kinc) were measured as previously described with the time scale of measurements being short enough that the effects of recycling were minimal (19, 25). Briefly, serum-starved cells were treated with labeled ligand at 37°C for five different time points from 0 to 7.5 min. Surface-bound and internalized fractions of labeled ligand were collected at five evenly spaced time points by acid-
stripping or NaOH solubilization, respectively, and the activities of these fractions were quantified using a 1470 Wizard Gamma Counter (PerkinElmer). From these data, \( k_e \) was calculated using a simple kinetic model, which included corrections for the effects of nonspecific binding and surface spillover. To determine the effects of inhibition of EGFR, MEK, or dynamin on \( k_e \), cells were treated for 1 h with 1 \( \mu \)M gefitinib (LC Labs, Woburn, MA), 10 \( \mu \)M U0126 (LC Labs), or 80 \( \mu \)M Dynasore (EMD Millipore, Billerica, MA) before \( k_e \) measurement. Stocks of gefitinib, U0126, and Dynasore were prepared in DMSO. Inhibitors were included with \(^{125}\)I-EGF during measurements. After \( k_e \) values were calculated for each independent experiment, statistical comparisons were performed with Graphpad Prism software using Student’s t-test to calculate \( P \) values.

**Measurement of TER.** Measurement of TER was performed as previously described (34). Briefly, control cells and Sec10-overexpressing cells were grown in triplicate on Transwell filters until TER levels reached steady state (3–5 days postconfluency). For \( H_2O_2 \) injury assays, the filters were incubated with freshly prepared 1 mM \( H_2O_2 \) in complete cell medium for 30 min and then changed back to normal complete cell medium for analysis of recovery. Half the filters were also incubated with 1 \( \mu \)M gefitinib for 1 h before and during the 30-min \( H_2O_2 \) treatment, with continued gefitinib treatment for 7 h afterward. At the indicated time points, TER was measured using an epithelial volt-ohmmeter (model EVOM; World Precision Instruments). Absolute TER values were determined by subtracting the TER of blank filters with medium from all samples, and the unit area resistance was obtained by multiplying the absolute TER by the effective surface area of the filter membrane. Statistical analysis was performed using SPSS software and included direct comparison of measured data at identical timepoints between two groups using Student’s t-test. To measure and compare epithelial recovery following injury, additional analysis included linear regressions of recovery times beginning at the nadir of TER (maximum injury) and statistical comparison of the calculated slopes (ohm \( \times \) cm\(^2\)/h).

**Photoablation of kidney epithelia in zebrafish.** All zebrafish experiments were approved by the Institutional Animal Care and Use Committee at Harvard. The ET(kt8:EGFP)sqet11–9 zebrafish line (henceforth referred to as ET11–9) was used for laser photoablation, which was recently described as a novel technique for studying renal injury in zebrafish (32). This zebrafish line has a part of the proximal tubule and distal tubule labeled with EGFP (6, 33). Embryos were produced by in-crossing heterozygous ET11–9 fish. They were injected at the 1-to-8 cell stage with 28 ng of sec10 morpholino (diluted in 0.5–1 nl of 100 mM KCl, 10 mM HEPES, and 0.1% phenol red at neutral pH), using the Drummond Nanoliter 2000 microinjector (WPI), or with equivalent amounts of standard control morpholino (Genetools). The sec10 morpholinos, the resulting phenotype, and their efficiency at downregulating sec10 have been described in detail in Fogelgren et al. (9). Given variable penetrance of injected morpholinos, sec10 morphant embryos were selected for photoablation injury based on the presence of the described “curly tail up” phenotype and the lack of general toxicity. Embryos were raised in E3 solution until 24 h postfertilization (hpf) and in PTU-containing E3 medium after 24 hpf (0.003% N-phenylthiourea, Sigma).

At 48 hpf, the embryos were anesthetized and immobilized in a 2% low melting point agarose as described by Vasilyev and Drummond...
(43). The 405-nm laser (100% intensity) of a Zeiss LSM5 laser scanning confocal microscope was used to induce segmental tubule injury within the GFP positive kidney epithelial segment, as described by Palmyre et al. (32). This wavelength allows one to induce injury specifically in the GFP-expressing cells (likely because of the GFP ability to absorb light in this wavelength). The amount of light exposure was adjusted to produce only a focal cell dropout in the control condition [26-μs pixel dwell time, 1 × 8 repeat (8 line average) in a line scan mode]. The amount of immediate injury was estimated by the percentage of GFP intensity reduction at a random cross-section line (by comparing GFP intensity profiles before and immediately after the 405-nm light exposure). The amount of eventual injury was estimated at 3 h postlaser ablation, as this time is generally sufficient for a full extent of kidney injury to develop (32). Confocal maximum intensity projections were generated and the average pixel intensity was measured for the injured segment as well as for the left and right intact epithelia adjacent to the injury site (these were averaged). The ratio of the average pixel intensity in the injured segment to the average pixel intensity in the “bracketing” intact segments was used to calculate the amount of epithelial dropout after photobleaching. The measurements of the initial injury were performed in Zeiss Pascal software. The measurements of the eventual injury were made in ImageJ (National Institutes of Health) and analyzed in Excel. Statistical analysis compared injury data from fish with Sec10-injected morpholinos (n = 8) vs. those injected with control morpholinos (n = 11) using a two-tailed t-test, unpaired with unequal variance.

RESULTS

Sec10 biochemically interacts with EGFR, and Sec10 overexpression results in more potent EGFR phosphorylation in response to EGF. We recently showed that Sec10 overexpression in renal tubule epithelial cells protects against oxidative injury and also enhances recovery of the epithelial barrier. Blocking the MAPK pathway in this system prevented Sec10’s protective effects (34). In polarized epithelial cells, the exocyst complex has been mostly associated with basolateral protein delivery (10, 22), although few studies have examined its possible role in receptor tyrosine kinase trafficking. Given that EGFR has been implicated in the recovery from acute kidney injury (3) and that EGF binding to EGFR is a well-described stimulus for activation of the MAPK pathway (2), we first tested for biochemical interactions between the exocyst and EGFR. A Sec10-GST fusion protein was purified on glutathione Sepharose and used as an affinity matrix for the purification of specific binding proteins from MDCK cell lysates. Western blot analysis identified EGFR as a protein pulled down with Sec10-GST, while no EGFR protein was detected in the control GST-only pull-down fraction (Fig. 1A).

Increased EGFR phosphorylation is accompanied by increased EGF binding and internalization. At least one report has implicated the exocyst in endocytosis (31), so we next investigated endocytosis of activated EGFR using radiolabeled EGF (19, 25). Serum-starved MDCK cells were treated with labeled 125I-EGF ligand at 37°C for up to 7.5 min, and both surface-bound and internalized 125I-EGF fractions were collected at regular time points. After calculating the rate constants (k1), we found EGF internalization to be increased in Sec10 overexpressing cells compared with control and Sec10 knockdown (KD) MDCK cells (Fig. 2A). This increase in EGF internalization was prevented by the
addition of gefitinib (aka Iressa), an inhibitor of EGFR tyrosine kinase activity (Fig. 2B); the addition of U0126, an inhibitor of MEK (Fig. 2C); as well as the addition of Dynasore, a cell-permeable small molecule inhibitor of dynamin-induced endocytosis (Fig. 2C).

Increased p-ERK in Sec10-overexpressing cells is due to increased signaling by EGFR. Gefitinib inhibits the EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme, preventing downstream activation of the MAPK signal transduction cascade (27). To test if the increased sensitivity of EGFR was the mechanism behind the increased p-ERK levels we had previously reported in Sec10-overexpressing cells (34), we grew control and Sec10-overexpressing MDCK cells for 1 wk on Transwell filters. Under basal conditions, we then added gefitinib at 1 µM for 1 h, which reduced the higher p-ERK levels measured in Sec10-overexpressing cells down to levels equal to control MDCK cells (Fig. 3).

Inhibition of EGFR signaling promotes injury and inhibits recovery of renal tubule cells following H2O2 exposure. We previously measured the degree of injury following H2O2 treatment and the time it took for the injured epithelial cells to recover their barrier integrity using TER as a marker. We found that overexpression of Sec10 significantly increased active ERK levels, decreased the degree of injury as measured by TER, and increased the rate of epithelial recovery following H2O2-induced injury. If U0126 was added in addition to the H2O2, Sec10-overexpressing cells recovered at the same rate as control cells, indicating that recovery was mediated through the MAPK pathway (34). Here we took a similar approach, but this time used gefitinib to evaluate EGFR’s contribution to Sec10’s protective role. In the absence of gefitinib, the severity of H2O2-induced injury (the nadir of TER) was greater in the control than the Sec10-overexpressing cells (Fig. 4A). Also, the timing of the nadir of TER was shortened in Sec10-overexpressing cells, indicating epithelial recovery began sooner. These observations were similar to what we previously showed (34). If gefitinib was added in addition to the H2O2, the severity of injury was greater in both cell lines, although Sec10 overexpression was still protective (Fig. 4A). Interestingly, the TER nadir was similar in the gefitinib-treated Sec10-overexpressing and untreated control cells (Fig. 4A), suggesting that EGFR signaling mediates the entire protective effect of Sec10 overexpression. The epithelial recovery rate from the TER nadir to the first point of significant increase was then determined using linear regression. The rate of recovery was decreased in both the Sec10-overexpressing and control cells following the addition of gefitinib (compared with untreated cells), but only reached statistical significance in the control cells (Fig. 4B).
Sec10 inhibition worsens damage in a zebrafish model of acute renal injury. For in vivo evaluation of Sec10’s protective role against injury in renal tubules, we utilized a newly developed zebrafish model of acute kidney injury as described by Palmyre et al. (32). This assay takes advantage of the ET(krt8:EGFP)sqet11-9 zebrafish line, which has a part of the proximal tubule and distal tubule labeled with EGFP. To inhibit Sec10 in these fish, we used microinjections of previously validated antisense morpholinos targeting sec10 (9). The sec10 knockdown embryos were selected based on the presence of tail curvature (9) and the lack of general toxicity. At 48 hpf the embryos were anesthetized and immobilized in agarose (43). With confocal microscopy, a 405-nm laser (100% intensity) was used to induce segmental tubule injury within the GFP-positive kidney epithelial segment. This wavelength allows one to induce injury specifically in GFP-expressing cells (likely because of the GFP ability to absorb light at this wavelength). The amount of immediate injury was estimated by the percentage of GFP intensity reduction at a random cross-section line (by comparing GFP intensity profiles before and immediately after the 405-nm light exposure) and was not significantly different in the Sec10 (n = 8) and control morphants (n = 11) (P = 0.16, data not shown). Additionally, the amount of eventual injury was estimated at 3 h post laser ablation, a time that is generally sufficient for the full extent of kidney injury to develop (32). For this measurement, we compared the average pixel intensity in the injured segment to the average pixel intensity in the “bracketing” intact segments. This method can be used because injured epithelial cells that undergo necrosis or apoptosis end up permanently losing their GFP fluorescence, as opposed to surviving cells that recover their preinjury fluorescence. After three h, renal tubule cell injury was significantly greater in sec10 morphants compared with control zebrafish (P = 0.034; Fig. 5, A and B). These data supported our in vitro findings that Sec10 expression in renal tubular epithelial cells is protective against cell damage.

DISCUSSION

Our goal in this study was to determine the mechanism by which exocyst Sec10 overexpression protects against oxidative injury in renal tubule cells. We previously showed that Sec10 overexpression increased MAPK signaling in MDCK cells, which protected the cells against oxidative injury by H2O2 (34). Given the role of the exocyst in trafficking proteins to the basolateral membrane in polarized epithelial cells (10, 22), we hypothesized that the exocyst regulates trafficking of signaling proteins upstream of ERK in the MAPK pathway. We focused on EGFR because of recent studies showing that EGFR is the most upregulated receptor tyrosine kinase in response to renal ischemia and reperfusion injury. Additionally, pharmacologic and genetic inhibition of EGFR in injured kidney proximal tubules markedly delayed cell proliferation and renal structural and functional recovery (3). Indeed, when screening for Sec10-binding proteins with GST pull-down assays from MDCK lysates, we detected a biochemical interaction between EGFR and the exocyst. We then measured levels of EGFR activation in response to EGF and found there was more phosphorylated EGFR in Sec10-overexpressing cells compared with control cells with the same EGF treatments.

At least one study has described a role for the exocyst in endocytosis (31), and EGFR endocytosis upon binding to EGF contributes to subsequent MAPK activation (19). By tracking internalization of radiolabeled EGF ligand, we discovered that the increase in MAPK activation in Sec10-overexpressing cells is likely due to increased EGFR endocytosis. Following the addition of gefitinib, the increase in EGF internalization, the increase in MAPK signaling, and the protective effect on membrane integrity of Sec10 overexpression all returned to levels seen in control MDCK cells.

Like other members of the receptor tyrosine kinase (RTK) superfamily, EGFR dimerizes (or forms heterodimers with other members of the ErbB family) and its kinase is activated upon ligand binding. Also like many other RTKs, the bound EGFR dimer then rapidly undergoes endocytic trafficking to early endosomes. There it either releases its ligand and gets recycled back to the plasma membrane, or it can remain ligand bound and gets degraded after shuttling through the late endosome to the lysosome (48). There is an established connection between EGFR endocytosis and ERK activation. For example in at least some cell settings, expression of a dominant negative form of dynamin (K44A) reduced EGFR endocytosis and...
investigating if culture model, we performed an in vivo functional study by signaling and exocyst recruitment.

The assembly of the exocyst complex in response to EGF binding of Exo70 to other exocyst components and promoted of ERK (35). Exo70 phosphorylation by ERK enhanced the component Exo70 was recently shown to be a direct substrate Sec10-overexpressing cells (34). Interestingly, the exocyst Sec10-overexpressing cells is because at the time of injury the reason that we see both protection and enhanced recovery in proximal tubule EGFR and MAPK signaling. One possible recovery following pharmacologic and genetic inhibition of ERK phosphorylation in Sec10-overexpressing cells.

increased EGFR endocytosis is the mechanism for increased identified as a regulator of endocytosis (31), suggesting that may allow chemical manipulation of exocyst activity by targeting upstream regulators of the complex assembly. With this goal in mind, it would be of great benefit to initiate screens to identify potential pharmacological modifiers of exocyst assembly and activity.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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