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Exocyst proteins in cytokinesis
Regulation by Rab11

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The Exocyst is an octameric protein complex comprised of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 subunits.[1,2] This complex was first identified in budding yeast where it acts to target vesicles to the bud tip and the cleavage furrow.[3] Here, we show that all Exocyst subunits are required for cytokinesis in mammalian cells. We further show that a subset of Exocyst proteins are differentially regulated by Rab11, consistent with recent studies implicating Rab11 vesicles in Exocyst protein trafficking.

Introduction

The Exocyst is thought to act as a tethering complex acting prior to fusion of secretory vesicles with the plasma membrane. First identified in yeast, the Sec15 subunit is anchored to the incoming secretory vesicle by interaction with the GTPase Sec4. The plasma membrane contains Sec3 and Exo70 which are thought to act as "spatial landmarks" for the arrival of the secretory vesicle, by forming an intact complex and bridging the 2 membranes.[4,5] An important question concerns how this complex is assembled: in mammalian cells, it has been proposed that some subunits (Sec10, Sec15, Exo84) "ride" on the vesicle and assemble with the other subunits at the plasma membrane.[6] However, studies in yeast suggest that all the subunits are associated with the vesicles.[5,7] Consistent with the latter model, recent studies using live-cell imaging of fluorescently tagged Sec8 revealed that this protein trafficked to the plasma membrane on vesicles.[8] This study also revealed a high degree of colocalization between tagged-Sec8 and recycling endosomes (marked by Rab11), but not other post-Golgi containers. Such observations led Rivera-Molina and Toomre to suggest that Exocyst-vesicles emanate from recycling endosomes.[8]

There is good evidence for a role for the Exocyst in cytokinesis. In *S. cerevisiae,* Exocyst components are localized to the site of cytokinesis and their depletion impairs cell cleavage. (Wang, 2002 #4555; TerBush, 1996 #2052) In *Drosophila*, a screen for cytokinesis mutants identified Sec5,[9] and Exocyst proteins have been identified in midbodies from mammalian cells.[10] Sec5, Sec8, Sec10, and Exo70 have been shown to be required for mammalian cell cytokinesis, as knockdown of these subunits results in a significant delay in abscission and an increased frequency of binucleate cells.[11] These phenotypes parallel those observed upon knockdown of Rab11 or disruption of Rab11/FIP3 interaction,[12] and we have previously shown that Rab11/FIP3 mediates an interaction between recycling endosomal-derived vesicles and the Exocyst.[13] Further supporting evidence is provided by the observation that distinct Exocyst complex subunits interact with a range of different trafficking proteins important for cytokinesis; e.g., Rab11 and Sec15,[14] Arf6 and Sec10,[15] and Sec5 and Exo84 with RalA and RalB.[16]

In this study, we show that all Exocyst subunits are required for cytokinesis, as knockdown of any component individually results in an increased frequency of binucleate cells. All subunits with the exception of Sec5 and Exo84 exhibit partial co-localization with Rab11; consistent with this, we found that knockdown of Rab11 also depleted Sec3, Sec8 Sec15, and Exo70, but not Exo84 or Sec5. Collectively, these data reveal that although all Exocyst subunits are required for successful cytokinesis, and suggest that individual components of the Exocyst are distinctly regulated. Our data are also consistent with the idea that components of the Exocyst traffic from recycling endosomes.

Results and Discussion

All Exocyst subunits are required for cytokinesis

Previous studies have reported that some of the Exocyst subunits are required for cytokinesis, notably Sec5, Sec8, Sec10,[11] and in a separate study, Exo70.[13] We examined the effect of knockdown of every Exocyst subunit in HeLa cells using siRNA SmartPools (**Fig. 1A**) and found that depletion of every subunit individually resulted in an increase in the frequency of binucleate cells, indicating that all subunits are required for cytokinesis. The frequency of binucleate cells observed upon Exocyst knockdown was similar to that observed when Rab11 was depleted.[12]

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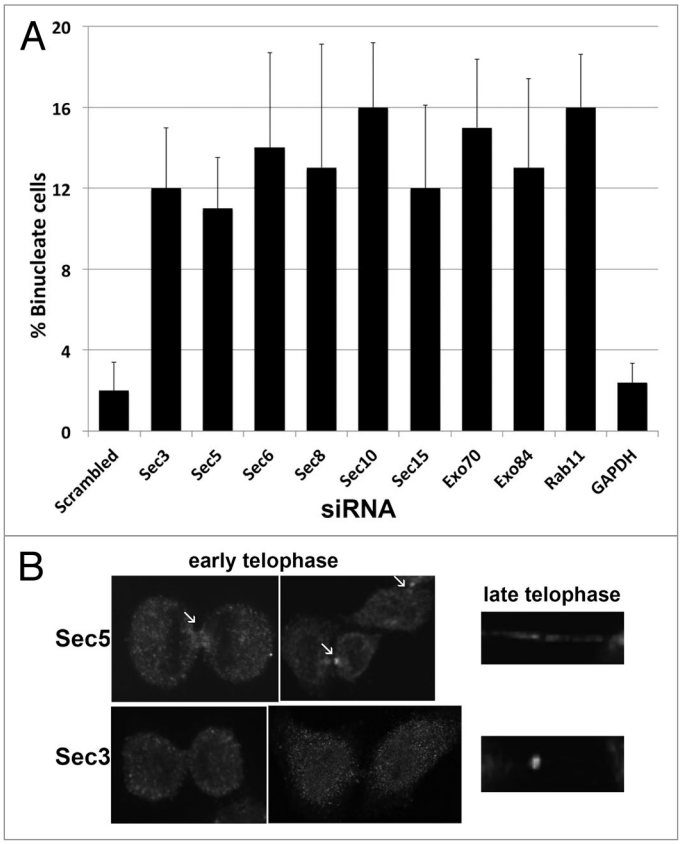

Figure 1. Exocyst proteins are required for cytokinesis. (**A**) HeLa cells were transfected with an siRNA SmartPools designed to knockdown the proteins shown on the *x*-axis, or with a scrambled siRNA SmartPool control, then incubated for 48 h after transfection before fixation and staining with DAPI and anti-tubulin antibodies. The frequency of binucleate cells was determined; shown is the data from a typical experiment, repeated 3 times with similar results, and is the mean and s.d. from 200 cells per condition. Immunoblot analysis (not shown) revealed knockdown of the proteins in question by at least 65% in each experiment (see also **Figure 2**). (**B**) HeLa cells were fixed and stained with antibodies against Sec 5 or Sec 3 as indicated. The arrows indicate cleavage furrows. Shown at right are magnified midbodies in late telophase.

Localization of Exocyst components during cytokinesis

We localized each component of the Exocyst complex during cytokinesis. Sec3, Sec6, Sec8, Sec10, Sec15, and Exo70 were observed in multiple punctae throughout the cytoplasm, and did not appear to be enriched in the furrow region until, late in telophase, when these subunits were observed in the characteristic midbody ring.[17] By contrast, we observed that Sec5 (**Fig. 1B**) and Exo84 (not shown) were enriched in the furrow early in telophase; as telophase continued, these subunits appeared throughout the bridge (**Fig. 1B**, insert at right).

Coordinate regulation of Exocyst components by Rab11

Studies have recently revealed a significant overlap of Sec8 with Rab11.[8] We observed that upon depletion of Rab11[12,13] using siRNA, levels of Sec3, Sec8, Sec15, and Exo70 consistently declined (**Fig. 2**); by contrast levels of Sec5 or Exo84 were

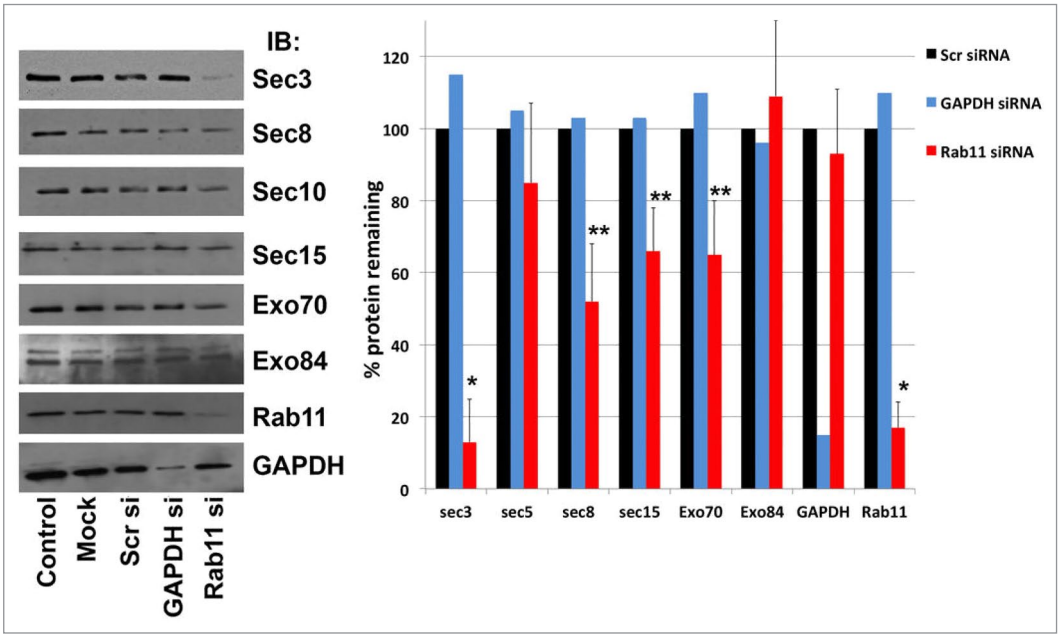

Figure 2. Exocyst subunits are differentially regulated by Rab11. HeLa cells were transfected with siRNA against Rab11, GAPDH, scrambled siRNA or transfection reagent only (mock) and 48 h later lysates prepared and immunoblotted for the proteins indicated. The bar graph shows quantification of 4 experiments of this type in which the % of a given protein remaining is compared between scrambled controls (set at 100%) and either GAPDH or Rab11 knockdowns. Significant differences are shown by * $P < 0.01$ and ** $P < 0.05$.

unaffected. Depletion of Rab35, another GTPase proposed to modulate membrane trafficking in cytokinesis was without effect on any Exocyst polypeptide levels (data not shown). These data are congruent with recent studies which revealed that fluorescent protein-tagged Sec8 trafficked on vesicles derived from recycling endosomes, and are also consistent with experiments in yeast which suggest that Sec8 resides on transport vesicles heading for the plasma membrane. The notion that multiple components of the Exocyst complex may traffic on vesicles is consistent with the data shown in **Figure 2**.

Summary

We show that all Exocyst components are required for cytokinesis in mammalian cells, but that these proteins exhibit differential localization patterns; Sec5 and Exo84 populate the growing furrow during early telophase, whereas other exocyst components do not. Future work should seek to establish whether these differential localization patterns are modulated by depletion of individual Exocyst components. By contrast, Sec3, Sec6, Sec8, Sec10, Sec15, and Exo70 appear as ring like structures in late telophase[17]which could not be observed for Exo84 and Sec5 (this study). Sec3, Sec6, Sec8, Sec10, Sec15, and Exo70 are coordinately regulated, as depletion of Rab11 (but not Rab35) selectively decreased expression of these proteins. These data are consistent with the idea that Rab11 is required for the trafficking of certain Exocyst components, and supports published work[8] on the role of Rab11 in the trafficking of Sec8. We propose that the traffic of the Exocyst, controlled by Rab11, plays a major role in cytokinesis.

Materials and Methods

Antibodies, DNA, and Reagents

Antibodies against the Exocyst components were as described.[13,17] siRNAs targeting the different Exocyst subunits were purchased as SmartPools from Dharmacon and used as described by the manufacturers.

Cell culture, transfection and imaging

HeLa cells were cultured, transfected, and processed for immunofluorescence as described.[12,13,17]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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