phosphorylation or other signals may represent parallel mechanisms that let myosin V know when it approaches active synaptic sites.

Interestingly, the E3 ubiquitin ligase Highwire is important for maintaining neuromuscular junctions in *Drosophila* (Wu et al., 2005). Could it, like Dma1, be involved in the release of a myosin V cargo from the membrane? It would also be interesting to test whether cargo adaptor degradation occurs to release microtubule motors, which, like yeast Myo2, are required for long-distance transport.

In summary, Yau et al. (2014) have discovered a novel mechanism to release cargoes from the class V myosin Myo2 through ubiquitination and proteolytic degradation of adaptor proteins. The Dma1 ubiquitin ligase is required to release more than one class of organelles from Myo2-based transport, suggesting that adaptor degradation could be a general mechanism of cargo release. Because of its potential for global relevance to the release of any cargo/motor interaction, this paper deserves a careful read, and the proposed mechanism merits further investigation in other biological systems.

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Spindlegate: The Biological Consequences of Disrupting Traffic

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The function of membrane trafficking during mitosis has become the focus of increasing interest. In this issue of *Developmental Cell*, Hehnly and Doxsey (2014) provide new insight into the role that endosomes play during spindle assembly.

Proper formation and precise positioning of the mitotic spindle are critical for successful asymmetric cell division. Here, mitotic spindle assembly initiates in prophase when the centrosomes start nucleating microtubules. Upon nucleation, the plus-end aster microtubules grow and extend toward the cell cortex (Lu and Johnston, 2013). As mitosis continues, the spindle is positioned by cortical polarity cues so that the cleavage furrow will bisect the central spindle during cytokinesis. Proper assembly of the spindle is critical for these events. Although membrane trafficking has been shown to affect the mitotic spindle and its position in the cell (Ai et al., 2009; Zhang et al., 2008), the precise mechanisms regulating these events are unknown. Work by Hehnly and Doxsey (2014) in this issue of *Developmental Cell* provides insight into these mechanisms by providing evidence for the requirement of Rab11 endosomes and their contents during spindle assembly.

Rab11 has primarily been studied as a regulator of membrane traffic. Rab11 regulates the recycling of endocytosed proteins to and from the plasma membrane or Golgi (Grant and Donaldson, 2009). Although these events have been shown to occur in numerous cell types and tissues during interphase, the surprise was that membrane trafficking was also required during key events in animal cell mitosis (Albertson et al., 2005). Since then, numerous studies in animal cells have documented the importance of membrane trafficking during cell division (Montagnac and Chavrier, 2008).

The Rab11 homolog, RAB-11, was first shown to be required during cell division in the *Caenorhabditis elegans* embryo (Skop et al., 2001). Subsequently, RAB-11 was shown to play a role in regulating aster microtubule size, spindle alignment, and the dynamic morphology of endoplasmic reticulum (ER) during mitosis. During metaphase, the reduced aster microtubule size in RAB-11-depleted embryos prevents many of the plus-end microtubules from making contact with the cell cortex, thus leading to spindle alignment defects. Depletion of RAB-11 also led to ER defects, suggesting that proteins, which regulate astral microtubule length, might be processed in the ER and

trafficked via RAB-11 endosomes (Zhang et al., 2008). In recent years, research has focused on the role of Rab11 and its effector molecule, Rab11-FIP3 (FIP3), during cytokinesis. Here, Rab11 and Rab11-FIP3 (FIP3) are thought to deliver components necessary for abscission (Wilson et al., 2005).

The observation that Rab11 plays a necessary role in microtubule aster size, spindle alignment, and cytokinesis suggests that Rab11-associated endosomes are spatially and temporally regulated during mitosis. However, we know little about what the Rab11 endosomes are doing or whether they contain factors important for these events. Results from the Doxsey laboratory (Hehnly and Doxsey, 2014) raise interesting possibilities for how the spatial regulation of spindle assembly is accomplished (Figure 1).

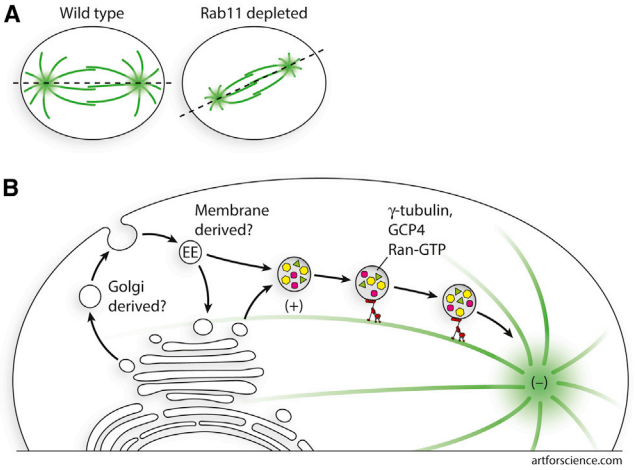

Figure 1. Model for Rab11 in Spindle Assembly and Alignment
(A) Depletion of Rab11 leads to reduced astral microtubule size and spindle orientation defects. (B) Rab11 endosomes transport spindle assembly factors along microtubules toward the spindle pole. The contents of Rab11 endosomes could come from the Golgi or via the plasma membrane and early endosome (EE) pathways. Figure by Adam Steinberg (http://www.artforscience.com).

To determine whether Rab11 functions during spindle assembly, the authors first confirmed that Rab11 localized to the spindle poles in HeLa cells. Next, by monitoring the localization of GFP-Rab11 endosomes during nocodazole and cold treatments, the authors tested whether the localization of Rab11 endosomes to the spindle and spindle poles was dependent on the polymerization state of the microtubules. Here, Rab11 endosomes dissociated from the spindle poles in both conditions and only remained associated with the spindle following cold treatment, suggesting that dynamic microtubules are necessary for Rab11 localization to the poles. This assay led to the conclusion that Rab11 endosomes associate with polymerized microtubules, probably for transport purposes.

Given previous work, Rab11 endosomes were hypothesized to transport necessary components for microtubule nucleation (Zhang et al., 2008), so the authors set out to determine what factors Rab11 endosomes contain, utilizing a sucrose step gradient to biochemically separate the proteins from membranes isolated from GFP-FIP3-expressing HeLa cells. The prominent proteins that cofractionated with the endosomes were γ-tubulin, GCP4, α-tubulin, and Ran-GTP, all factors involved in microtubule nucleation and spindle assembly. Intriguingly, more Rab11 and γ-tubulin associated with mitotic endosomes than with interphase endosomes, suggesting that mitotic membranes are able to recruit these microtubule-nucleating factors. Lastly, Rab11 and Rab11 endosomes were found to be necessary to localize other key spindle assembly factors to centrosomes, including pericentrin, EB1, and RanBP2. Overall, this study suggests that the recycling endosome and its contents play a necessary role in the targeting of factors required for spindle organization and microtubule dynamics.

One question remained as to whether the recycling endosomes were transported along the microtubules themselves. To determine this, several straightforward experiments were conducted. Recycling endosome dynamics following nocodazole treatments and subsequent drug washouts were observed over time. Here, Rab11 endosomes associated with newly formed microtubules and in structures called microtubule clusters, which the Doxsey laboratory described previously. These clusters of endosomes, microtubules, and γ-tubulin were transported vectorially toward the spindle poles, a unique observation for Rab11 endosomes. In the absence of dynein, the Rab11 endosomes remained stationary following nocodazole treatment and subsequent drug washout, indicating that Rab11 endosomes traffic toward the spindle poles.

Taken together, these results demonstrate a necessary role for Rab11 endosomes and their trafficking during spindle assembly. The identification of some of the contents of the Rab11 endosomes is especially exciting because this sheds light on previous phenotypes observed when Rab11/RAB-11 was depleted. Here, microtubule asters shrunk and spindle orientation and cytokinesis failures were observed (Ai et al., 2009; Skop et al., 2001; Zhang et al., 2008), which Hehnly and Doxsey confirm in this study (Hehnly and Doxsey, 2014). It is possible that Rab11 endosomes contain factors necessary for all of these mitotic events. However, some fundamental questions remain regarding where and when these factors are packaged into endosomes for these events during the cell cycle. The spindle pole and spindle assembly factors could originate via endocytosis of membrane through the early endosome pathway, via the Golgi, or via both as a means of temporal and spatial regulation. Given the finding that EB1, a plus-end tip binding protein, is mislocalized in Rab11 small interfering RNA interference-treated cells, it is possible that Rab11 endosomes also mediate the targeting of other factors to the plus ends of microtubules or out to the cell cortex. Given the cytokinesis failures observed in Rab11-depleted cells (Hehnly and Doxsey, 2014; Wilson et al., 2005) and *C. elegans* embryos (Zhang et al., 2008), Rab11 endosomes could contain factors necessary for the maintenance of the spindle midzone during cytokinesis. Here, KIF23 (MKLP1/CHO1/ZEN-4) could be actively transported to the midzone via Rab11 endosomes during anaphase up until abscission. Lastly, the defects in ER morphology observed in RAB-11 *C. elegans* embryos (Zhang et al., 2008) could indicate that factors necessary for a proper microtubule-ER network are found in Rab11 endosomes. The spatial and temporal regulation of the

protein content of the recycling endosome during mitosis will uncover some interesting revelations.

For many years, membrane trafficking was thought to be suspended until the end of mitosis (Albertson et al., 2005). Over the past decade, research from numerous laboratories has shown how important mitotic membrane trafficking events are during cell division. The work by Hehnly and Doxsey (2014) highlights and reinforces the importance of membrane trafficking prior to cytokinesis, particularly in spindle assembly. How the spindle and membrane content of the cell work together is only just beginning to be understood. Future research will uncover the complexities of these processes and the importance and regulation of the protein content that resides in mitotic endosomes.

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