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MEMBRANE DYNAMICS

ER trailblazing by RAB10

> "phospholipid synthesis could be synchronized with ER dynamics"

The endoplasmic reticulum (ER) extends throughout much of the cytoplasm and is constantly remodelled through microtubule-guided extension and homotypic membrane fusion events. English and Voeltz have now found that the GTPase RAB10 mediates tubule extension and may help to localize phospholipid synthesis to these dynamic domains.

RABs provide specificity to membrane fusion events throughout the cell, and previous *in vitro* studies had suggested that they might also control ER homotypic fusion. English and Voeltz set out to test this and addressed whether this might require a specific RAB. Using *Xenopus laevis* egg extracts as a model for *in vitro* ER fusion, they first showed that pre-incubation with a RAB-specific GDP dissociation inhibitor (RABGDI) blocked fusion, confirming a role for RABs. They then devised an affinity purification strategy to isolate RABs from this extract through their binding to a GTP•agarose column. This strategy identified a few RABs, but of these candidates only RAB10 was found to be localized to the ER membrane.

Using time-lapse microscopy, the authors showed that tagged RAB10 localized to several domains of the ER, including the peripheral cisternae and tubules. Moreover, RAB10 was important for the normal ratio of ER cisternae to tubules; expression of a GDP-locked RAB10 mutant or siRNA-mediated depletion of RAB10 led to increased numbers of cisternae and fewer tubules. Disruption of RAB10 function (using the GDP-locked mutant or siRNA-mediated depletion) also reduced the number of ER tubule extension events and reduced the efficiency of ER tubule fusion. Moreover, consistent with the known role of microtubules in guiding ER dynamics, depolymerization of microtubules disrupted RAB10-driven ER extension events.

Closer examination revealed that RAB10 localized to a dynamic domain associated with the ER (that was distinct from the ER lumen). This localization was similar to that recently shown for the enzyme PIS, which catalyses the formation of phosphatidylinositol in the ER. Indeed, English and Voeltz showed that RAB10 and PIS colocalized to this dynamic domain, and using live imaging they observed that this domain frequently localized to the leading edge of extending ER tubules. If RAB10 function was blocked (again using the GDP-locked mutant or siRNA-mediated depletion of RAB10), the number of ER extension and fusion events was markedly reduced.

As several enzymes that synthesize phospholipids localize to the ER, the authors asked whether RAB10 colocalization with PIS is unique. They saw that another lipid-synthesizing enzyme, CEPT1 (choline and ethanolaminephosphotransferase 1; which is involved in phosphatidylethanolamine and phosphatidylcholine synthesis), also partitions with RAB10 and PIS to dynamic ER domains. Expression of the GDP-locked RAB10 disrupted the localization or dynamics of CEPT1 and PIS, respectively.

RAB10 thus acts as a guide to drive ER tubules along microtubules. The authors propose that phospholipid synthesis could be synchronized with ER dynamics, and an attractive possibility is that lipid synthesis in these domains could have implications for the delivery of lipids to particular sites in the cell. This RAB10 pathway seems to be independent of ER fusion events that are mediated by the atlastin GTPases, so ER dynamics may be orchestrated by several distinct mechanisms.

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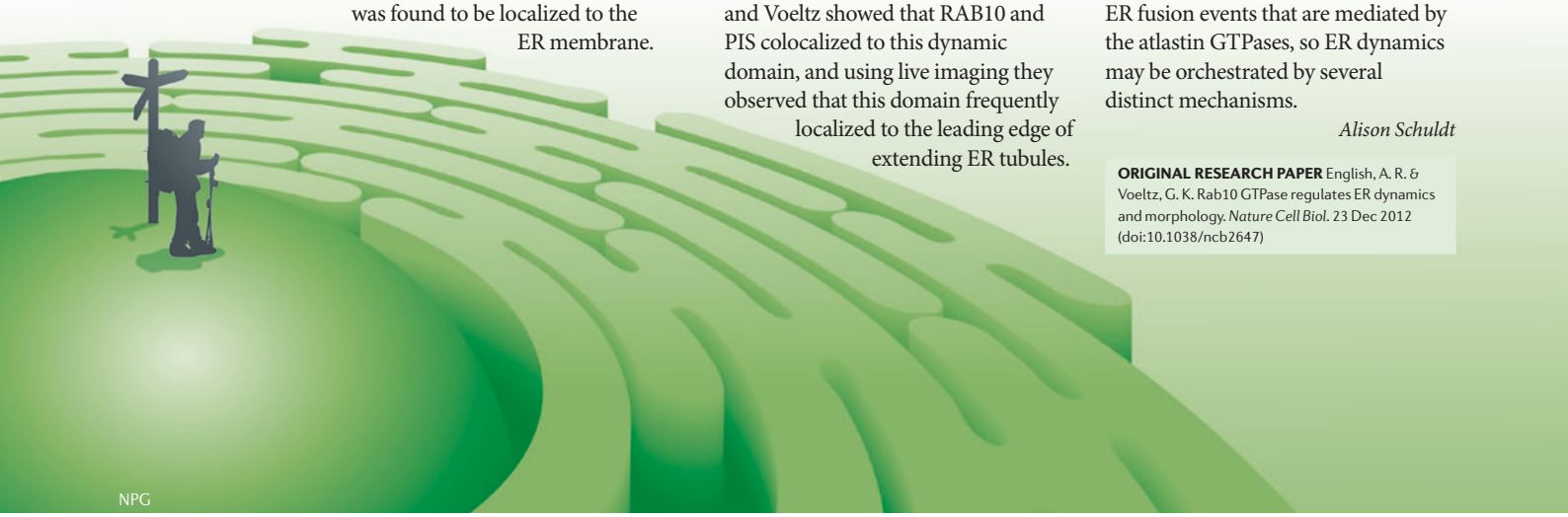

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