GNOM alone

Polarized vesicular trafficking is essential for a plethora of fundamental biological functions. In mammals, this includes processes as diverse as the maintenance of epithelial cell polarity, downregulation of cell-surface receptors and progress through key stages of development. In plants, by contrast, we know almost nothing about the significance of polarized transport. Now, a study by Geldner *et al.* (*Cell* **112,** 219–230 (2003)) provides evidence of a direct link between transport of auxin, an essential regulator of plant growth, and polarized membrane transport.

There have been some recent hints to suggest that polarized transport is essential for fundamental processes in plants. The first came from studies of auxin, which is actively transported throughout the plant via specific transporters that are organized in a polar distribution. One candidate transporter for auxin, PIN1, is continuously recycled through the endomembrane system, suggesting a potential requirement for polarized vesicle transport. The second hint came from analysis of the *Arabidopsis thaliana* protein, GNOM, a guanine nucleotide-exchange factor for ARF GTPases — essential regulators of vesicle trafficking in many organisms. Mutations in *GNOM* caused defects that were highly reminiscent of the effects of blocking auxin transport. At a sub-cellular level, PIN1 was mis-localized in *gnom* mutants. Furthermore, treatment with Brefeldin A (BFA), which is known to reversibly block vesicular trafficking steps by inhibiting the activity of specific ARF-GEFs, also disrupted auxin transport and resulted in the mis-localization of PIN1 to internal membranes. Despite this circumstantial evidence, however, no direct molecular link between the movement of auxin and polarized trafficking has been demonstrated.

Now, Geldner *et al.* provide direct evidence of such a link. Their first interesting observation was that GNOM is localized to endosomes. This was unexpected, as related GEFs from yeast and animals have only been associated with secretory transport routes. In addition, they found that the endosomal morphology of *gnom* mutants was significantly disrupted, suggesting an essential role of GNOM in the maintenance of endosomal integrity and function. Next, in an elegant series of experiments, the authors engineered a mutant form of GNOM that is fully functional, but resistant to the effects of BFA. This allowed them to specifically dissect out the function of GNOM from other BFA-sensitive trafficking steps. Crucially, when plants expressing the mutant protein were then treated with BFA, PIN1, but not other plasma membrane proteins, was correctly localized to the cell surface. This provides convincing evidence that GNOM directly regulates the continual polarized recycling of PIN1 and suggests that ARF-GEFs regulate specific membrane trafficking pathways in plants. In addition, Geldner *et al.* show that the block in auxin transport induced by BFA is restored in cells expressing BFA-resistant GNOM. The next step is to identify the particular ARF on which GNOM exerts its effects. Nevertheless, this study directly links a component of membrane traffic to the transport of auxin, highlighting the importance of polarized trafficking in fundamental aspects of plant biology.

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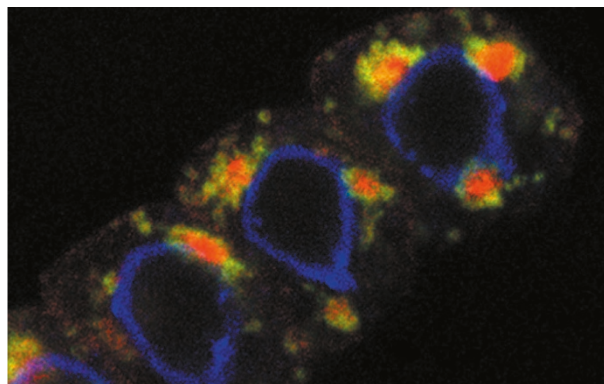

Figure 1 **GNOM redistributes to an endosomal compartment after BFA treatment. GNOM (red) and γ-COP (green) in *Arabidopsis* root cells after treatment with 50 μM BFA for 1 h. The nucleus is stained with DAPI (blue). Figure adapted from Geldner *et al.* © (2003) with permission from Elsevier Science.**

the absence of protease activity. To demonstrate this point, separase was overexpressed in cells arrested in metaphase. This resulted in release of Cdc14 from the nucleolus in a manner that was dependent on the FEAR pathway. To examine whether the protease activity of separase was important, the experiment was repeated with a ‘protease-inactive’ separase. Again, Cdc14 was efficiently released from the nucleolus. As sister chromatids remained associated when the protease-inactive separase was overexpressed, this argues that the ability of separase to promote mitotic exit is not a mere consequence of sister chromatid separation, in agreement with earlier work[7]. As securin can inhibit mitotic exit[6,11], it was important to exclude the possibility that overexpression of separase, active or inactive, did not pull securin away from another putative mitotic exit activator(s). To this end, Uhlmann and colleagues examined whether the protease-inactive separase can promote mitotic exit after the vast majority of securin was degraded, so that competition with other possible securin targets was not likely to be an issue. In this experiment, cells carrying a conditional separase allele, *esp1-1*, were synchronously released from G1 at the non-permissive temperature in the presence of either wild-type or protease-inactive separase, both expressed from the native separase promoter. The *esp1-1* mutant alone had very low levels of Cdc14 release that occurred only at late anaphase, presumably through the MEN pathway. By contrast, in the presence of either wild-type or protease-inactive separase, Cdc14 release was much more robust and occurred earlier in anaphase. Importantly, the effects of wild-type separase and that of the protease-inactive form were indistinguishable, suggesting that the proteolytic activity of separase is dispensable for promoting mitotic exit. There are, however, two caveats to this interpretation: first, the cumulative level of the separase proteins, namely the wild-type or protease-inactive form together with the product of the *esp1-1* allele, may still be higher than normal physiological levels. This could have resulted in the displacement of securin from other putative mitotic regulators, inadvertently resulting in their premature activation. Second, the experiment was performed in a strain that carried the *esp1-1* allele, the product of which was shown to have residual protease activity[12]. As separase is an essential protein, the experiment could not be easily done in a complete null background. Thus, it is possible that the protease-inactive form of separase stimulated the activity of the *esp1-1* product, perhaps by stabilizing it or by increasing its intranuclear concentration. However, the fact that protease-inactive separase was just as effective as the wild-type

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