MEMBRANE TRAFFICKING

One step becomes two

The fusion of viral envelopes with endosomal membranes and the release of nucleocapsids into the cytoplasm of host cells were thought to occur in a single step during viral infection. However, in *Nature Cell Biology*, Gruenberg and colleagues describe how this one step can be two.

Vesicular stomatitis virus (VSV) takes advantage of the endocytic pathway to infect host cells, and it was thought that nucleocapsid release occurs immediately after viral-envelope fusion with endosomal membranes, which is triggered by the acidic pH of these endocytic compartments. However, the authors found that viral fusion and viral infection (nucleocapsid

release into the cytosol and subsequent viral protein synthesis) are two distinct steps. Viral fusion was triggered before most virions reached late endocytic compartments — the drug-induced depolymerization of microtubules, which are required for transport from early to late endosomes, had no significant effect on viral fusion. By contrast, viral infection was affected by drug-induced microtubule depolymerization.

VSV infection, but not fusion, was also blocked by endocytosed antibodies against lysobisphosphatidic acid (LBPA), which selectively interfere with late endosome function. Together, these observations indicate that viral fusion occurs in multivesicular bodies (MVBs) — transport intermediates between early and late endosomes — and that viral envelopes fuse with intraluminal vesicles, rather than with the

endosomal membrane. Electron-microscopy studies confirmed that fusion released viral nucleocapsids into the intraluminal vesicles of MVBs, where they remained hidden, rather than into the cytosol. Viral infection, on the other hand, requires transport to late endosomes and the subsequent delivery of viral nucleocapsids to the cytosol.

Focusing on the latter step, Gruenberg and co-workers showed that, in addition to LBPA, the delivery of viral nucleocapsids to the cytosol depends on the putative LBPA effector Alix. This delivery also seems to be regulated by phosphatidylinositol-3-phosphate (PtdIns3P) signalling (PtdIns3P is abundant in endosomes) through the PtdIns3P-binding sorting nexin Snx16. They conclude that "...the nucleocapsid is exported into the cytoplasm after the back-fusion of internal vesicles with the limiting

PLANT CELL BIOLOGY

Close encounters

The plant hormone auxin controls many different aspects of plant growth and development. It mediates its downstream effects through changes in gene expression by targeting the Aux/IAA transcriptional repressors for ubiquitin-ligase-mediated degradation. Other aspects of auxin signalling, such as auxin perception, are less well understood. But the groups of Ottoline Leyser and Mark Estelle now report the remarkable finding that the F-box protein TIR1, which is part of the ubiquitin ligase complex SCF^{TIR1}, is an auxin receptor.

The two groups had previously shown that auxin promotes the interaction between Aux/IAA proteins and SCF^{TIR1}. Now, using pull-down assays, they have shown that the immunoprecipitated SCF^{TIR1} complexes retain the ability to interact with tagged Aux/IAA protein or peptide in an auxin-dependent manner — indicating that the auxin receptor must be present in the precipitate.

The Estelle group found that the effect of auxin was temperature independent. This, together with the continuous requirement of auxin for the Aux/IAA–SCF^{TIR1} interaction,

argued against the possibility that auxin regulates the interaction by causing a stable enzymatic modification of TIR1 or Aux/IAA. Instead, might auxin modulate this interaction by binding SCF^{TIR1} directly? Both groups carried out further pull-down assays, but in the presence of radioactive auxin. Radioactivity was retained in the presence of wild-type Aux/IAA protein, but not with a mutant that was unable to bind SCF^{TIR1}. Competitive binding studies with auxin and related compounds showed that auxin binding to SCF^{TIR1} was specific and dose dependent. They also confirmed that the auxin receptor must be localized to the SCF^{TIR1} complex.

As TIR1 is the only known auxin-specific subunit of the SCF^{TIR1} complex, the two groups tested whether auxin interacted directly with TIR1 — which it did. Leyser, Estelle and co-workers expressed TIR1 in two non-auxin-responsive systems (*Xenopus laevis* and insect cells), prepared cell extracts and used pull-down assays to show that heterologously expressed TIR1 and Aux/IAA proteins interacted in an auxin-specific and dose-dependent manner. However, a TIR1

mutant that lacked the F-box motif — which is required for binding the SCF protein SKP1 (ASK1 in *Arabidopsis thaliana*) — did not respond to auxin. So, auxin-induced binding of TIR1 to Aux/IAA requires the F-box motif. Whether this means that the deleted sequence directly mediates binding, or that the function of TIR1 as an auxin receptor requires its assembly into an SCF complex (or at least its interaction with SKP1), remains an open question.

The identification of TIR1 as an auxin receptor identifies a new mode of SCF regulation that might apply to some of the many uncharacterized SCFs in plants and animals. How auxin stimulates the interaction between SCF^{TIR1} and Aux/IAA is not clear — it could promote a conformational change in SCF^{TIR1} that favours Aux/IAA binding, or auxin might cooperatively bind both TIR1 and Aux/IAA. The authors also suggest that there are additional auxin receptors, including TIR1-related proteins and possibly an extracellular receptor.

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References and links

ORIGINAL RESEARCH PAPERS Dharmasiri, N. *et al.* The F-box protein TIR1 is an auxin receptor. *Nature* **435**, 441–445 (2005) | Kepinski, S. & Leyser, O. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* **435**, 446–451 (2005)

FURTHER READING

Dharmasiri, N. *et al.* Plant development is regulated by a family of auxin receptor F box proteins. *Dev. Cell* 2 June 2005 (doi:10.1016/S153458070500184X)