VIRAL TRANSMISSION

# Deadly contact

Transmission of the human T-cell lymphotropic virus type I (HTLV-I) seems to require cell–cell contact — the contribution to infection by cell-free HTLV-I virions is minimal. In their report in *Science*, Igakura *et al.* now show that HTLV-I is transmitted across cell–cell junctions after polarizing the cytoskeleton of the infected cell at sites of cell–cell contact.

The authors first looked at the distribution of the viral (Gag) core proteins and the glycoprotein envelope (Env) protein in isolated infected T cells, and in uninfected cells that had conjugated with HTLV-I-infected cells. After 40 minutes, they saw a strong polarization of both proteins from around the cell periphery in infected cells to the area of cell–cell contact in conjugates — a significant finding because the nucleocapsid p15 Gag protein is known to incorporate the retroviral genome into virions. In addition, another Gag protein, p19, was detected in the 'uninfected' cells of the conjugates, which might represent the initial establishment of HTLV-I infection.

Following on from the detection of p15 at the cell–cell contacts, Igakura *et al.* studied the localization of the HTLV-I genome. The HTLV-I nucleic acid was not polarized in single infected T cells, but it accumulated at cell–cell junctions of infected–uninfected conjugated cell pairs, similar to what was seen for the Gag and Env proteins. As was also seen for the Gag p19 protein, viral nucleic acid was later transferred to the 'uninfected' cell.

What is the cause of this asymmetrical localization? The authors noticed that polarized Gag proteins at the cell–cell junctions were frequently closely juxtaposed to a reorientated microtubule-organizing centre (MTOC). As nocodazole, which depolymerizes microtubules, inhibited the cell–cell accumulation and subsequent cell transfer of Gag, this implicates microtubule dynamics in the polarization of Gag. In addition, Igakura *et al.* showed that MTOC

PLANT DEVELOPMENT

# Channelling elongation

Everybody knows that to grow, plants need minerals and water from the soil, which they obtain through roots and root hairs. The formation of these structures requires cell expansion — by way of elongation — which, in turn, needs calcium ($Ca^{2+}$) acquisition. But, until now, what regulated the $Ca^{2+}$ influx wasn't so obvious. Research led by Liam Dolan's group, though, has pinpointed the production of reactive oxygen species (ROS) by an NAPDH oxidase in the activation of $Ca^{2+}$ channels in elongating root cells.

Because *Arabidopsis thaliana rhd2* mutants develop very short root hairs and stunted roots, and are defective in $Ca^{2+}$ uptake, the authors decided to clone the gene encoding RHD2. They found that the gene — *At5g51060* — had previously been defined as *Arabidopsis thaliana* respiratory burst oxidase homologue C (*AtrbohC*). Rather unsurprisingly, as implied by the name, the *At*rbohC protein and other *At*rbohs are homologous to the $gp91^{phox}$ subunit of the mammalian NAPDH oxidase that catalyses ROS production.

What, then, is the connection between RHD2/*At*rbohC and growth? ROS production was reduced by ~50% in root apices from *rhd2* mutants compared with wild-type apices. Normally, ROS are present as the root hair emerges as a bulge and further increase as the elongation rate goes up. Adding an inhibitor of NADPH oxidase to the apices of wild-type plants prevented ROS accumulation, the elongation of root-hair bulges and the extension rate of the primary root, thereby phenocopying the *rhd2* mutant.

The authors then tried the opposite approach. Could ROS applied to *rhd2*-mutant root-hair bulges induce root-hair growth? Indeed it could. Application of the most reactive ROS, hydroxyl radicals ($OH^{\bullet}$), to *rhd2*-mutant root-hair bulges restored root-hair growth, although the growth lacked the polarity found in wild-type hairs. Moreover, this was coincident with a rapid increase in the cytoplasmic levels of $Ca^{2+}$ ($[Ca^{2+}]_c$), which was blocked in the presence of 0.1 mM $Gd^{3+}$, a $Ca^{2+}$-channel antagonist.

These data implicated ROS in the increase of $[Ca^{2+}]_c$ by $Ca^{2+}$ influx, so the next step was to see if plasma-membrane $Ca^{2+}$ channels could be activated by ROS. Within a few minutes of $OH^{\bullet}$ treatment, a $Ca^{2+}$-permeable, inwardly rectifying, hyperpolarization-activated conductance was detected in protoplasts from the elongation zone epidermis. This was again blocked by 0.1 mM $Gd^{3+}$, which also decreased the root elongation rate, as did a $Ca^{2+}$ chelator. Because *rhd2* mutants and wild-type cells didn't differ significantly in their current amplitudes, the *rhd2* mutation seems not to affect the ROS-mediated channel sensitivity or the number of channel proteins. In root-hair apical spheroplasts, $OH^{\bullet}$ activated a $Ca^{2+}$-, $Ba^{2+}$- and $TEA^{+}$-permeable, inwardly rectifying, hyperpolarization-activated conductance.

So in protoplasts from the elongation zone epidermis and apical spheroplasts, ROS is involved in cell elongation by activating $Ca^{2+}$ channels. The influx of $Ca^{2+}$ is likely to modulate actin dynamics and other growth processes, and this mechanism could well extend to all plant cells. As the mammalian $gp91^{phox}$ is regulated by Rac, the authors propose that RHD2/*At*rbohC could be similarly controlled by Rac-like proteins in plants — ROPs.

*Katrin Bussell*

## References and links

**ORIGINAL RESEARCH PAPER** Foreman, J. *et al.* Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 2003 March 27 (DOI: 10.1038/nature01485)

**WEB SITE**

**Liam Dolan's laboratory:**
http://www.jic.bbsrc.ac.uk/science/cdb/dolanwebpage.htm

polarization occurred within the infected cell, rather than towards it.

So, when an HTLV-I-infected T cell contacts another cell, microtubule rearrangements in the infected T cells occur, and the viral genome is subsequently transferred to the recipient cell. The identity of the molecules that initiate contact and polarization is unknown; the Env protein is one candidate for fusion, being the only HTLV-I protein that is expressed on the outside of the infected cell, but HTLV-I also upregulates the expression of some adhesion molecules that could favour cell–cell transmission. Other viruses that depend on cell contact for transmission, or that are lymphotropic — such as HIV-1 — might similarly subvert normal T-cell physiology to propagate between cells.

*Katrin Bussell*


**References and links**

**ORIGINAL RESEARCH PAPER** Igakura, T. *et al.* Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science Express* 2003 February 13 (DOI: 10.1126/science.1080115)



ORGANELLE TRAFFICKING

# Breakdown to arrive

When you're travelling to a particular destination, there's always the risk that mechanical breakdown will thwart your arrival. However, Weisman and colleagues now report in *Nature* that transport-machinery breakdown can actually be essential for cellular organelles to reach their final destination.

For yeast vacuole inheritance to occur correctly, vacuoles must be transported from the mother cell to the growing bud. The yeast class V myosin Myo2 moves organelles along actin to different destinations during the cell cycle, and previous studies indicated that the yeast vacuole membrane protein Vac8 is needed for Myo2's role in vacuole inheritance. However, Vac8 and Myo2 had not been shown to interact directly. Weisman and co-workers therefore began by identifying Vac17 as the vacuole-specific receptor for Myo2. However, Vac17 is not a membrane-bound protein, so how does it link Myo2 to vacuoles?

The authors showed that a Vac17–Vac8 interaction is the missing link and that the regions of Vac17 that interact with Vac8 and Myo2 are distinct. Vac17 can therefore interact with both proteins simultaneously — Vac17 links Myo2 to Vac8 and, as a result, to the vacuole membrane.

Vacuole inheritance was found to be blocked by mutations that disrupted either Myo2–Vac17 or Vac17–Vac8 interactions, and the authors found that this block resulted in Vac17 accumulation. Furthermore, they showed that Vac17 protein levels and the levels of vacuole-associated Vac17 change during normal cell-cycle progression. The levels of vacuole-localized Vac17 increase on formation of the nascent bud, decrease with increasing bud size, and decrease further on vacuole-inheritance completion. So, are these changes in Vac17 levels due to increased synthesis, decreased breakdown, or both? And could changes in Vac17 levels control vacuole inheritance?

Sequence analysis allowed the authors to identify a predicted PEST sequence in Vac17, which is a signal for rapid protein degradation. They found that deletion of PEST resulted in increased Vac17-ΔPEST levels, although Vac17-ΔPEST supported normal vacuole inheritance. These results indicate that, in this case and in vacuole-inheritance mutants, Vac17 accumulation is a result of defective Vac17 degradation, rather than because of a significant increase in Vac17 synthesis.

Using immunofluorescence, Weisman and co-workers showed that, in *VAC17-ΔPEST* mutants, Vac17-ΔPEST accumulates in the bud, whereas, in vacuole-inheritance mutants, Vac17 accumulates in the mother cell. These results support the idea that Vac17 degradation occurs after it has arrived in the bud.

When vacuole inheritance is complete, vacuoles are localized near the centre of the bud, and the authors found that, in the *VAC17-ΔPEST* mutant, several vacuoles were localized to the mother–bud neck. Using time-course experiments, they showed that removal of the PEST sequence stabilizes the Myo2–Vac17 interaction, which causes the vacuoles to move 'backwards' from the bud centre to the mother–bud neck.

Together, these results support a model in which newly synthesized Vac17 binds to Vac8 and Myo2 in the mother cell to form the Myo2–Vac17–Vac8 transport complex, which moves the vacuole along actin to the bud. In the bud, Vac17 is degraded in a PEST-dependent manner, which releases Myo2 from the vacuole and results in the vacuole being deposited near the centre of the bud. So, transport breakdown doesn't always hinder arrival after all!

*Rachel Smallridge*


**References and links**

**ORIGINAL RESEARCH PAPER** Tang, F. *et al.* Regulated degradation of a class V myosin receptor directs movement of the yeast vacuole. *Nature* 2003 February 16 (DOI: 10.1038/nature01453)

**FURTHER READING** Catlett, N. L. & Weisman, L. S. Divide and multiply: organelle partitioning in yeast. *Curr. Opin. Cell Biol.* **12**, 509–516 (2000)

**WEB SITE**

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http://bcaws.biochem.uiowa.edu/weismanlab/index2.html