membrane of late endosomes, and that this process is controlled by the phospholipids LBPA and PtdIns(3)P and their effectors".

This work has shown that VSV entry into cells requires two steps, not one. And, although it is important to remember that this route is not followed by all viruses, it might have been hijacked by other pathogenic agents such as anthrax toxin. In the future, it will be interesting to elucidate the molecular mechanisms that underlie the back fusion of intralumenal vesicles with the limiting membrane of MVBs, which will provide further insights into this increasingly versatile compartment.

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References and links

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SIGNAL TRANSDUCTION

A change in taste

To unleash their full phosphorylating potential, kinases themselves need to be phosphorylated — frequently by autophosphorylation of a residue in the so-called activation loop. Lochhead *et al.* studied two dual-specificity Tyr-phosphorylation-regulated kinases (DYRKs) in a bid to find out precisely how this happens, and found that the kinases initially phosphorylate themselves on Tyr, but later turn their attention to Ser/Thr substrates. Further details of this intricate change in taste are reported in *Cell*.

The authors focused on two *Drosophila melanogaster* DYRKs — dDYRK2 and minibrain (MNB) — and identified Tyr358 in dDYRK2 and Tyr326 in MNB as the only residues that were phosphorylated when the recombinant proteins were expressed in insect cells. Furthermore, phosphorylation of dDYRK2 on Tyr358 was necessary for it to then show Ser/Thr activity towards an exogenous substrate.

DYRK Tyr phosphorylation is thought to occur by autophosphorylation. If this occurs in *trans* (that is, is intermolecular), kinase-inactive forms of the proteins would still be expected to be tyrosine phosphorylated. However, kinase-inactive dDYRK2 or MNB weren't phosphorylated when expressed alone or with their wild-type counterpart, and they couldn't phosphorylate a peptide containing their active loop. This indicates that DYRK phosphorylation occurs through a *cis* (intramolecular) mechanism.

Initially, Lochhead *et al.* carried out *in vitro* kinase assays by incubating dDYRK2 or MNB with γ-^{32}P-ATP to try to identify the Tyr-phosphorylated residues. Instead, they found that only Ser and Thr residues were autophosphorylated. Was this because the Tyr autophosphorylation sites were saturated? To address this, the kinases were treated beforehand with a Tyr phosphatase — this showed that they were resistant to dephosphorylation, with only a 50% reduction after 60 min (probably indicating that the activation loop Tyr was inaccessible). This coincided with a 50% reduction in Ser/Thr kinase activity, confirming the requirement of activation loop Tyr phosphorylation for Ser/Thr activity.

Notably, the DYRKs that were dephosphorylated could not rephosphorylate on Tyr. So Tyr autophosphorylation seemed to be a 'one-off' event. By studying the translation of dDYRK2 *in vitro*, Lochhead *et al.* found that the levels of protein expression increased alongside the amount of Tyr phosphorylation (both were detected after 20 min). They then saw that, although both wild-type and kinase-inactive dDYRK2 co-purified with ribosomes during the *in vitro* translation process, only the wild-type form autophosphorylated on Tyr; translation of all the canonical kinase subdomains was required for this to occur.

Finally, Lochhead *et al.* carried out drug-sensitivity assays. Purvalanol A and TBB are known inhibitors of DYRKs, but only Purvalanol A inhibited dDYRK2 autophosphorylation during *in vitro* translation. This difference in sensitivity of the two drugs implies that the mature, folded DYRK structure differs from the transitional intermediate emerging from the ribosome. Supporting the concept that autophosphorylation of the DYRK activation loop Tyr occurs once only and is mediated by a transition intermediate, Purvalanol-A-treated dDYRK2 translation products were incapable of autophosphorylating on Tyr.

So intramolecular autophosphorylation of DYRK activation loop Tyr residues occurs as a 'one-off' event during protein folding before the nascent polypeptide disengages from the ribosome, but only its mature counterpart can phosphorylate substrates on Ser/Thr. Proof indeed that tastes change over time. The authors speculate that the existence of a transitional intermediate that autophosphorylates is probably not unique to DYRKs.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Lochhead, P. A. *et al.* Activation loop autophosphorylation is mediated by a novel transitional intermediate form of DYRKs. *Cell* **121**, 925–936 (2005)

WEB SITE

Vaughn Cleghon's laboratory: http://www.beatson.gla.ac.uk/cleghon.htm