

also involved in the flattening of the Golgi cisternae; the depletion of myosin XVIIIa both disrupts these straight trajectories and induces the collapse of the Golgi complex around the centrosome¹⁶. Following myosin XVIIIa-dependent budding, Miserey-Lenkei *et al.* have now shown that myosin II promotes transport carrier fission². Subsequently, myosin VI intervenes to regulate the dynamics of Golgi-derived transport carriers and possibly coordinate their passage through the perinuclear recycling endosomal compartments, which is an intermediate step for some of the carriers moving towards the plasma membrane¹⁷. Myosin VI is recruited to the post-Golgi recycling endocytic compartments by Rab8, through optineurin¹⁷. Although the precise role of myosin VI in this pathway remains to be defined, it might be required both for cargo sorting at recycling endosomes and for delivery of basolateral cargo to the plasma membrane¹⁷. In yeast, multiple Rabs, but one myosin, coordinate in TGN-to-plasma membrane transport

(Fig. 2); the Rab Ypt32p activates Myo2p to move vesicles along actin cables, and is then exchanged for the Rab Sec4p, which further supports Myo2p mobility and regulates vesicle docking in the bud¹⁸.

What is the nature and extent of the interaction between Rab6–myosin II and other fission machinery at the Golgi? Inhibition of Rab6 and/or myosin II does not completely block exit from the Golgi complex²; this indicates that additional fission factors intervene in the formation of transport carriers in the Rab6-dependent pathway. Among these factors, the dynamin-driven machinery that cooperates with cortactin and actin to mediate membrane fission at the TGN, as well as PKD (protein kinase D) and BARS (brefeldin-A-dependent ADP-ribosylated substrate), represent possible candidates¹⁷. If, and how, Rab6–myosin II interfaces with dynamin or with other fission-promoting proteins at the TGN remains an important area for further investigation.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Stenmark, H. *Nat. Rev. Mol. Cell Biol.* **10**, 513–25 (2009).
2. Miserey-Lenkei S. *et al. Nat. Cell Biol.* **12**, 645–654 (2010).
3. Grigoriev, I. *et al. Dev. Cell* **13**, 305–314 (2007).
4. Girod, A. *et al. Nat. Cell Biol.* **1**, 423–430 (1999).
5. Martinez, O. *et al. Proc. Natl Acad. Sci. USA* **94**, 1828–1833 (1997).
6. Martinez, O. *et al. J. Cell Biol.* **127**, 1575–1588 (1994).
7. Mallard, F. *et al. J. Cell Biol.* **156**, 653–664 (2002).
8. Hill, E., Clarke, M. & Barr, F. A. *EMBO J.* **19**, 5711–5719 (2000).
9. Coutelis, J. B. & Ephrussi, A. *Development* **134**, 1419–1430 (2007).
10. Yumura, S. *J. Cell Biol.* **154**, 137–146 (2001).
11. Araki, N., Hatae, T., Furukawa, A. & Swanson, J. A. *J. Cell Sci.* **116**, 247–257 (2003).
12. Lenz, M., Morlot, S. & Roux, A. *FEBS Lett.* **583**, 3839–3846 (2009).
13. Römer, W. *et al. Cell* **140**, 540–553.
14. Deretic, D. *et al. J. Cell Sci.* **108**, 215–224 (1995).
15. Foth, B. J., Goedecke, M. C. & Soldati, D. *Proc. Natl Acad. Sci. USA* **103**, 3681–3686 (2006).
16. Dippold, H. C. *et al. Cell* **139**, 337–351 (2009).
17. De Matteis, M. A. & Luini, A. *Nat. Rev. Mol. Cell Biol.* **9**, 273–284 (2008).
18. Bielli, P., Casavola, E. C., Biroccio, A., Urbani, A. & Ragnini-Wilson, A. *Mol. Microbiol.* **59**, 1576–1590 (2006).
19. Schlager, M. A. *et al. EMBO J.* **29**, 1637–1651.

Tagging the dead: a bridging factor for *Caenorhabditis elegans* phagocyte receptors

Rachael Rutkowski and Anton Gartner

Recognition of apoptotic cells by phagocytic cells in *Caenorhabditis elegans* has been something of a mystery. A secreted transthyretin-like protein, TTR-52, has been identified as a bridging molecule between apoptotic cells and CED-1 on the phagocytic cells that engulf them.

The process of apoptosis not only involves the death of a cell, but also its engulfment and degradation by phagocytic cells. In mammals, several receptors and ligands have been identified that regulate a key first step in this process — the recognition of a dying cell by phagocytes^{1,2}. Apoptotic cells have been hypothesized to present ‘eat me’ signals to surrounding phagocytes. So far the only ‘eat me’ signal that has been extensively characterised is the phospholipid phosphatidylserine, normally localized to the inner leaflet of the plasma membrane but exposed on the

external surface of dying cells¹. In mammals, several receptors recognize exposed phosphatidylserine including the phosphatidylserine receptor, the T-cell immunoglobulin and mucin-domain-containing molecules TIM-1 and TIM-4, stabilin-2, brain-specific angiogenesis inhibitor BAI-1, integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, and the receptor tyrosine kinase MER^{1,3}. Some of these receptors such as the phosphatidylserine receptor, TIM-4 and BAI-1 bind to phosphatidylserine directly, whereas others, such as integrins and MER, recognize phosphatidylserine through bridging molecules such as milk fat globule-EGF factor 8 (MFG-E8) or growth arrest specific gene 6 (Gas-6; ref. 2). Surprisingly, less is known about how engulfing cells recognize apoptotic cells in *C. elegans*. On page 655 of this issue, Wang *et al.* show that the *C. elegans*

secreted transthyretin-like protein, TTR-52, acts as a bridging molecule between the ‘eat me’ signal phosphatidylserine on the surface of the apoptotic cell and the CED-1 receptor on the membrane of the engulfing cell⁴.

Among the molecular players implicated in PS recognition in mammals, the *C. elegans* genome only encodes homologues of the phosphatidylserine receptor and integrin- α , termed PSR-1 and INA-1, respectively. Both proteins mediate efficient engulfment of apoptotic cells^{5,6}, but the role of *psr-1* appears to be minor, and has been contested^{7,8}. Similarly to the mammalian phosphatidylserine receptor, PSR-1 directly binds to exposed phosphatidylserine. INA-1-mediated recognition of dying cells requires the activity of the scramblase, SCRM-1, which allows the translocation of phosphatidylserine from the inner to the outer

Rachael Rutkowski and Anton Gartner are in the Wellcome Trust Centre for Gene Regulation and Expression, University of Dundee, Dundee DD1 5EH, UK.
e-mail: r.j.rutkowski@dundee.ac.uk
DOI:10.1038/ncb2077

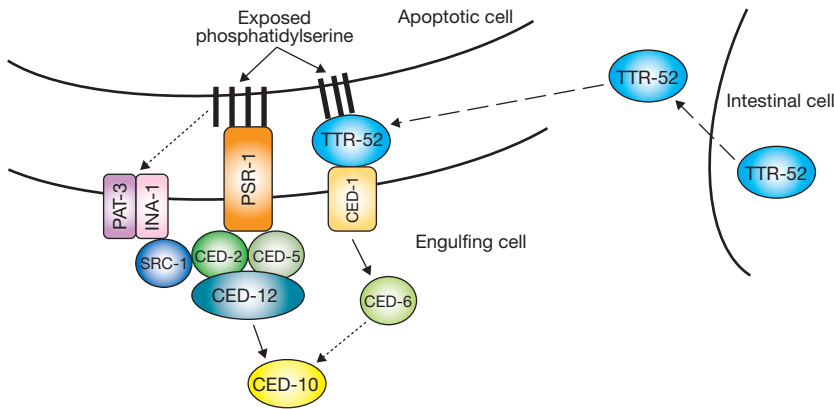


Figure 1 TTR-52 is a bridging molecule that mediates the recognition of phosphatidylserine by the CED-1 phagocytic receptor in *C. elegans*. TTR-52 is expressed in intestinal cells, from which it is secreted and can then bind to exposed phosphatidylserine on the surface of dying cells. The bound TTR-52 recruits CED-1 at the surface of the nearby engulfing cell, leading to the activation of the CED-1/CED-6 engulfment pathway and ultimately CED-10/Rac-1. Other known phosphatidylserine recognition pathways in *C. elegans* could also act in parallel. PSR-1 can bind phosphatidylserine directly, interacts with both CED-2 and CED-5, and acts through this parallel engulfment pathway. The integrins α /INA-1 and β /PAT-3 also respond to exposed phosphatidylserine and activate the CED-2/CED-5/CED-12 pathway through the *C. elegans* SRC-1 Src kinase, which can bind both INA-1 and CED-2.

leaflet of the plasma membrane. However, it is not clear how INA-1 recognizes phosphatidylserine. Both PSR-1 and INA-1 signal to the CED-2 (CrkII), CED-5 (Dock180), CED-12 (ELMO) and CED-10 (Rac GTPase) engulfment pathway. Another *C. elegans* death receptor, CED-1 (LRP1/MEGF10), functions in a parallel engulfment pathway with the CED-6 (GULP) adaptor molecule and the CED-7 (ABCA1) ABC transporter and converges with the first pathway by activating CED-10 (ref. 9). The *C. elegans* Frizzled homologue MOM-5 was recently proposed to be a co-receptor for CED-1 (ref. 7).

Wang *et al.* have shown that CED-1 also recognizes phosphatidylserine⁴. However, this recognition requires TTR-52, the first bridging molecule mediating apoptotic corpse recognition in *C. elegans* to be identified (Fig. 1). A mutant allele (*sm211*) of a transthyretin-like encoding gene, *ttr-52*, was identified in a classic genetic screen for mutants that had an increased number of apoptotic corpses in late embryogenesis because of a delay in corpse engulfment. Genetic epistasis experiments show that the *ced-1 ttr-52* double mutant phenotype is not stronger than either single mutant, whereas the engulfment defect of *ced-2* is enhanced by the *ttr-52* mutant, thus demonstrating that *ttr-52* acts in the *ced-1* pathway, probably in parallel with the *ced-2* pathway. The biological functions of transthyretin-like proteins are unknown but most, including

TTR-52, are predicted to be secreted. Wang *et al.* found that GFP- and mCherry-tagged TTR-52, expressed in embryos, was present on the surface of apoptotic cells, and that their localization was dependent on the presence of the secretory signal peptide of TTR-52. A membrane-tethered TTR-52 fusion protein failed to cluster around dying cells, irrespective of whether it was expressed in dying or engulfing cells. Similarly, a TTR-52 *sm211* mutated protein fused to GFP was not secreted and did not localize around dying cells. The expression of all three mutant proteins that failed to cluster around dying cells also failed to rescue the engulfment defect of the *ttr-52* mutant, indicating that TTR-52 secretion is important for proper engulfment.

As TTR-52 is secreted, Wang *et al.* determined in which cells TTR-52 is expressed. They generated a transcriptional reporter using the *ttr-52* promoter to drive the expression of mCherry and observed mCherry expression specifically in intestinal cells, indicating that once TTR-52 is expressed, it is secreted from the intestine and is able to find and bind to apoptotic cells.

As TTR-52 acts in the same pathway as CED-1, Wang *et al.* tested whether TTR-52 and CED-1 are present together on dying cells. CED-1-GFP and TTR-52-mCherry colocalized on 69% of dying cells and time-lapse imaging showed that TTR-52 binds to a dying cell prior to CED-1. *ttr-52* mutants exhibited a

decrease in CED-1 localization to dying cells, consistent with the idea that TTR-52 localization on dying cells is key to the recruitment of phagocytes. Wang *et al.* showed that TTR-52 interacts with the extracellular domain of CED-1 in a GST pulldown experiment and also immunoprecipitates with CED-1-GFP from worm extracts.

To identify the signal mediating TTR-52 localization to dying cells, the authors performed a genetic screen. This screen identified an allele of *tat-1*, which encodes an aminophospholipid translocase. *tat-1* mutants show exposed phosphatidylserine on the surface of all cells, not just apoptotic cells¹⁰. Worms carrying the *tat-1* allele identified in the screen showed TTR-52 localization to all cells, consistent with the notion that TTR-52 may bind phosphatidylserine. A yeast cell-based assay was used to determine whether exogenous TTR-52 could bind phosphatidylserine. In wild-type yeast, phosphatidylserine is localized to the inner leaflet of the plasma membrane, but yeast *cho1* mutants are defective in phosphatidylserine synthesis and in this strain, phosphatidylserine-binding proteins localize throughout the cytosol. As expected for a phosphatidylserine-binding protein, TTR-52 localized to the membrane in wild type and is found in the cytosol in *cho1* mutants. This assay was then used to identify the phosphatidylserine-binding domain of TTR-52. Mutating amino-acid residues 50–55 to alanines prevented phosphatidylserine binding in yeast and expression of the same mutant in worms failed to rescue the engulfment defect of *ttr-52* mutants — although the mutant protein was secreted it did not cluster around apoptotic cells. *In vitro*, recombinant TTR-52 bound preferentially to phosphatidylserine, compared with other phospholipids.

The findings by Wang *et al.* describe the first bridging molecule to be discovered in *C. elegans* that can mediate the recognition of apoptotic cells by engulfing cells, and identify a mechanism by which CED-1 recognizes apoptotic cells. As noted by the authors, the engulfment phenotype of *ttr-52* mutants is weaker than that observed in *ced-1* mutants, implying that there are probably more bridging molecules still to be discovered. Alternatively, CED-1 may respond to other 'eat me' signals apart from phosphatidylserine. Although CED-1 and phosphatidylserine are conserved components of the engulfment recognition pathway, it is unclear whether the involvement of TTR-52 or a related

family protein is also conserved and it will be interesting to determine whether related proteins have a similar role in mammals.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Fadeel, B. & Xue, D. *Crit. Rev. Biochem. Mol. Biol.* **44**, 264–77 (2009).
2. Kinchen, J. M. *Apoptosis* doi:10.1007/s10495-010-0509-5 (2010).
3. Zhou, Z. *Dev. Cell* **13**, 759–60 (2007).
4. Wang, X. *et al. Nat. Cell Biol.* **12**, 655–664 (2010).
5. Wang, X. *et al. Science* **302**, 1563–6 (2003).
6. Hsu, T. Y. & Wu, Y. C. *Curr. Biol.* **20**, 477–86 (2010).
7. Cabello, J. *et al. PLoS Biol.* **8**, e1000297 (2010).
8. Zullig, S. *et al. Curr. Biol.* **17**, 994–9 (2007).
9. Kinchen, J. M. *et al. Nature* **434**, 93–9 (2005).
10. Darland-Ransom, M. *et al. Science* **320**, 528–31 (2008).

Nuclear transport receptor goes moonlighting

Oliver J. Gruss

The importin- β -like transport receptors and RanGTP govern selective transport of proteins into the nucleus. It has now been shown that importin- β 2 (alternatively called transportin1) also selectively targets the motor protein Kif17 to primary cilia. In analogy to the nucleus, RanGTP in the intraciliary compartment mediates dissociation of Kif17 from its transport receptor and thereby completes import.

The import of proteins from the cytoplasm into the nucleus is receptor-mediated and signal-dependent. A short stretch of basic amino acids was the first nuclear localisation signal (NLS) to be fully defined, almost 25 years ago¹. On page 703 of this issue, Verhey and colleagues find that targeting of the microtubule motor Kif17 to primary cilia depends on a ciliary localization signal (CLS) that, surprisingly, shares similarities with NLSs². Both signals can use the same transport receptor, which either transfers proteins across the nuclear envelope or targets them to the intraciliary compartment. Nuclear transport and targeting of proteins to primary cilia therefore share common molecular determinants (Fig. 1) and might have evolved from the same ancestral targeting machinery.

Many eukaryotic cells develop non-motile, primary cilia that function as cellular ‘antennae’, transducing mechanical or chemical stimuli into intracellular signals that control developmental pathways. The assignment of several diseases — now commonly called ‘ciliopathies’ — to the compromised functions of ciliary gene products has re-established primary cilia as a focus of cell biological research (for a review, see ref. 3). In general, cilia are generated around the unique microtubule array of the axoneme, which initially assembles and elongates from the basal body. Bidirectional transport along

axonemal microtubules, termed intraflagellar transport (IFT)⁴, helps to set up and maintain the ciliary membrane as a specialized plasma membrane domain protruding from the cell body. Consistently with this, kinesin motors and cytoplasmic dyneins are found in cilia, as part of a unique functional proteome of the intraciliary compartment⁵. Accumulating evidence indicates that membrane traffic controlled by Rab proteins governs sorting of respective membrane proteins to the ciliary membrane^{6,7}, but the general concepts and molecular mechanisms to explain how soluble proteins from the cytoplasm become localized to the intraciliary compartment are scarce.

The data of Verhey and colleagues provide new insights into this process². The authors first investigated which primary structural elements in Kif17, a kinesin that functions in IFT, mediate ciliary targeting of the motor. They expressed Kif17 tagged with a yellow fluorescent protein variant (mCitrine) in different cell lines that generate primary cilia and monitored the characteristic accumulation of the motor at the distal tip of primary cilia. Through this, they identified a short carboxy-terminal sequence in Kif17 that is required for ciliary targeting, and therefore called a ‘CLS’. This sequence contains several basic amino acids and is similar to an NLS. Amazingly, the Kif17 CLS also specifically mediates binding to the nuclear import receptor importin- β 2, which confirms the similarity between a CLS and an NLS. The identification of an NLS-like CLS seems paradoxical at first. How can a signal, which is similar to an NLS, also work as a selective sorting signal for ciliary targeting? Why does Kif17 not accumulate in the nucleus? Verhey and

colleagues show that the motor domain makes the difference: a motor-less, C-terminal fragment of Kif17 did end up in the nucleus, but retargeted to primary cilia when fused to even a non-ciliary motor domain. Targeting of Kif17 to primary cilia therefore involves a ‘bipartite’ ciliary import signal. The microtubule motor domain provides the first and currently uncharacterised part; the signal herein might be a conserved signature of the primary structure, or the association state of the motor protein, such as dimerization or microtubule binding. The other part of the signal is related to an NLS sequence and requires the function of importin- β 2. Consistently with this, indirect immunofluorescence microscopy detected importin- β 2 at the proximal side of primary cilia.

Interestingly, an 18 amino acid ciliary targeting signal (CTS) was recently identified within the cytoplasmic tail of the ciliary membrane protein, fibrocystin. In this case, most mutations of the critical residues in the CTS interfered with both proper ciliary targeting and Rab8 interaction, indicating that the CTS is responsible for the interaction of fibrocystin with Rab8, and thus mediates ciliary targeting. However, although mutations in a few basic amino acids in the C terminus of the protein led to mistargeting, it did still interact with Rab8. This suggests that additional targeting factors must exist⁸, which recognize basic amino acids, very much like several nuclear import receptors. One might therefore speculate that importins work together with Rab proteins to target ciliary membrane proteins. This might be analogous to the targeting of proteins to the inner nuclear membrane, which depends on

Oliver J. Gruss is in the Zentrum für Molekulare Biologie, ZMBH, DKFZ ZMBH Alliance, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany.
e-mail: o.gruss@zmbh.uni-heidelberg.de
DOI:10.1038/ncb2076