

TRAPPING Rab18 in lipid droplets

Francesca Zappa¹, Rossella Venditti¹ & Maria Antonietta De Matteis^{1,2}

A number of membrane trafficking components are associated with lipid droplets (LDs) and/or are involved in their biogenesis. In this issue of *The EMBO Journal*, Li *et al* (2017) show that the mammalian TRAPP II (TRANSPORT Protein Particle) complex acts as an LD-associated GEF for Rab18, thereby regulating LD homeostasis.

See also: C Li *et al* (February 2017)

Far from being simple storage structures, lipid droplets (LDs) are highly dynamic organelles that are involved in several functions. Testifying to their dynamic nature, several membrane trafficking components are linked to LDs. These include ARF guanine nucleotide exchange factors (GEFs), COPI components, and several Rab GTPases. Despite the fact that almost 40 different Rab GTPases have been found to associate with LDs, functional data regarding their role in LD biogenesis are available for only a few of them (Rab1, Rab5, Rab7, Rab8a, Rab32, Rab40c, and Rab18).

Rab18 associates with LDs, and it has been proposed to play a role in establishing the connection/exchange between the endoplasmic reticulum (ER) and the LD (Ozeki *et al*, 2005). The most convincing evidence supporting a role for Rab18 in LD homeostasis comes from the observation of an accumulation of enlarged lipid droplets in fibroblasts from patients affected by Warburg Micro syndrome, a neurological syndrome caused by mutations in Rab18, in Rab3GAP1/Rab3GAP2, which works as a GEF for Rab18, or in TBC1D20, a Rab18 GTPase-activating protein (GAP) (Handley *et al*, 2015).

Rab18 also has a role in the maintenance of the ER and the Golgi complex (Gerondopoulos *et al*, 2014), and the activity

of Rab3GAP1/Rab3GAP2 is required for the association of Rab18 with the endoplasmic reticulum (ER) (Handley *et al*, 2015). Therefore, the question remains whether the role of Rab18 in LD homeostasis is mediated by its function at the ER, or whether the activation of Rab18 can be regulated locally at the level of the LD by an LD-associated Rab18 GEF.

In this issue of *The EMBO Journal*, Li and co-workers provide an answer to this question. By adopting a combination of siRNA and gene-editing experiments, they demonstrate that the mammalian TRAPP (TRANSPORT Protein Particle) complex, and in particular TRAPP II, acts as a LD-associated GEF for Rab18.

TRAPP, first identified in yeast almost 20 years ago, is a multisubunit modular complex consisting of core and peripheral subunits that is highly conserved from yeast to mammals. Several TRAPP complexes have been isolated in yeast (TRAPP I, TRAPP II, TRAPP III, and, very recently, TRAPP IV) (Kim *et al*, 2016; Lipatova *et al*, 2016) sharing the same core subunits but containing distinct peripheral subunits. Only two TRAPP complexes, TRAPP II and TRAPP III, have been described to date in mammals (Scrivens *et al*, 2011; Bassik *et al*, 2013).

The different TRAPP complexes in yeast take part in distinct trafficking events ranging from ER-to-Golgi transport (TRAPP I), late transport steps in the Golgi (TRAPP II), and autophagy (TRAPP III and TRAPP IV). The picture is far less defined in mammals where TRAPP II has been found to play a role in intra-Golgi and/or Golgi-to-PM transport (Yamasaki *et al*, 2009) and in ciliogenesis, while TRAPP III has been shown to control ER-to-Golgi transport (Scrivens *et al*, 2011), autophagy (Imai *et al*, 2016; Lamb *et al*, 2016), human papilloma virus infection, and

ricin toxicity (Bassik *et al*, 2013; Kim *et al*, 2016). In addition, a role in the ER export of fibrillar procollagen has been shown for TRAPP C2, a component that is shared by TRAPP II and TRAPP III and the product of the gene mutated in the spondyloepiphyseal dysplasia tarda (Venditti *et al*, 2012) (Fig 1).

Initially proposed to act as a “passive” tethering factor, it was later shown that TRAPP complexes possess active “catalytic-like” roles by acting as GEFs for GTPases of the Ypt/Rab family in both yeast and mammals (Kim *et al*, 2016).

In yeast, TRAPP I, TRAPP III, and TRAPP IV can act as GEFs for Ypt1p (the homologue of Rab1) but sustain the role of Ypt1p in distinct processes, that is, ER-to-Golgi trafficking (TRAPP I) and autophagy (TRAPP III, TRAPP IV), thus indicating the existence of pathway-specific GEFs activating the same GTPase (Kim *et al*, 2016). Thus, the emerging scenario is that it is the GEF function of the TRAPP III/IV complex that confers specificity on the activation of the same GTPase in different processes. The yeast TRAPP II complex instead acts as a GEF for Ypt31/32 (the homologues of Rab8/11). In mammals, both TRAPP II and TRAPP III have been shown to act as a GEF for Rab1. With their findings, Li *et al* (2017) now demonstrate that the same TRAPP II complex acts as a GEF for another Rab GTPase, Rab18, at the level of LDs. The authors immunoprecipitated TRAPP II and TRAPP III using complex-specific subunits (TRAPP C9 and TRAPP C12, respectively) and tested the GEF activity on recombinant Rab proteins *in vitro*. They found that the TRAPP II exchange activity on Rab1 and Rab18 is comparable, while TRAPP III failed to activate either of them. The functional relevance of the GEF activity of TRAPP II on

¹ Telethon Institute of Genetics and Medicine, Pozzuoli, Italy. E-mail: dematteis@tigem.it

² Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy
DOI 10.15252/embj.201696287 | Published online 27 January 2017

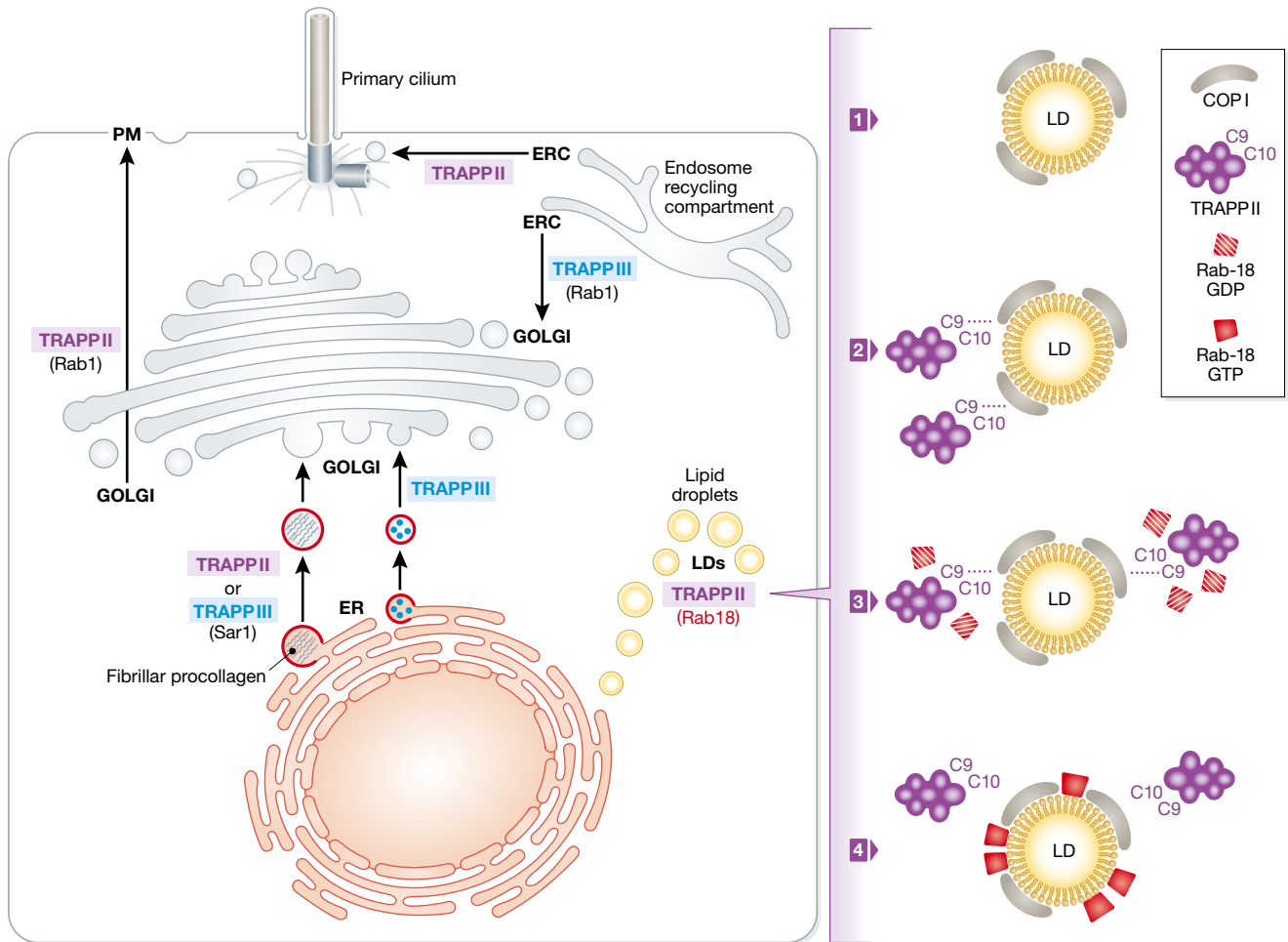


Figure 1. Sites of action of TRAPPII and TRAPPIII complexes in mammalian cells.

Two TRAPP complexes have been identified in mammalian cells, TRAPPII and TRAPPIII, which share common core subunits (TRAPPC1, TRAPPC2, TRAPPC3, TRAPPC4, TRAPPC5) but differ in the peripheral subunits: TRAPPII contains TRAPPC9 and TRAPPC10, while TRAPPIII contains TRAPPC8, TRAPPC11, TRAPPC12, and TRAPPC13. The scheme illustrates the different steps along the anterograde and retrograde membrane trafficking pathways that are under control of each TRAPP complex. In anterograde trafficking, TRAPPC2 is required for the ER export of fibrillar procollagens indicating a cargo-specific role for TRAPPII and/or TRAPPIII in this step, TRAPPIII is required for ER-to-Golgi trafficking, while TRAPPII controls intra-Golgi and/or Golgi-to-plasma membrane trafficking and the trafficking of ciliary proteins from endosomes to the primary cilium. In the retrograde pathway, TRAPPIII controls the trafficking from endosomes to the Golgi complex of different cargoes including ATG9, a key component of the autophagic machinery. The report by Li *et al* (2017) highlights a novel site of action for TRAPPII at lipid droplets. Also indicated are the Rabs activated by TRAPPII and by TRAPPIII at their different sites of action.

Rab18 is highlighted by the very similar LD phenotype (i.e., enlarged LDs) caused by either Rab18 or by TRAPPC9 depletion.

The site of action of TRAPPII as a GEF for Rab18 is at the LD where TRAPPII is recruited upon lipid load via its interaction with COPI (in particular through the interaction of the TRAPPII-specific subunit TRAPPC9 with γ -COP). These data therefore also highlight a new role for the LD-associated COPI complex in addition to its proposed role in mediating the establishment of tubular connections between the ER and the LD (Wilfling *et al*, 2014).

While identifying a new molecular pathway fundamental for LD biogenesis, the

work by Li *et al* (2017) also poses new questions: what is the relationship between the two Rab18 GEFs, that is, Rab3GAP1/Rab3GAP2 and TRAPPII? Do they intervene at different stages of LD biogenesis? Is LD-associated TRAPPII responsible for the activation of other LD-associated Rabs?

Finally, the data also provide the first example of a site of action of a mammalian TRAPP complex that is distinct from the endomembrane system, that is, in a lipid monolayer. It will be interesting to test in the future whether TRAPP is also found to participate in the biogenesis/assembly of other cellular structures not necessarily bound by a lipid bilayer.

Acknowledgements

We thank Cathal Wilson for critical reading of the manuscript. M.A.D.M. acknowledges the support of Telethon, the Italian Association for Cancer Research (AIRC, grant IG2013_14761), and European Research Council Advanced Investigator grant no. 670881 (SYSMET).

References

Bassik MC, Kampmann M, Lebbink RJ, Wang S, Hein MY, Poser I, Weibezahn J, Horlbeck MA, Chen S, Mann M, Hyman AA, Leproust EM, McManus MT, Weissman JS (2013) A systematic mammalian genetic interaction map reveals pathways underlying ricin susceptibility. *Cell* 152: 909–922

- Gerondopoulos A, Bastos RN, Yoshimura S, Anderson R, Carpanini S, Aligianis I, Handley MT, Barr FA (2014) Rab18 and a Rab18 GEF complex are required for normal ER structure. *J Cell Biol* 205: 707–720
- Handley MT, Carpanini SM, Mali GR, Sidjanin DJ, Aligianis IA, Jackson IJ, FitzPatrick DR (2015) Warburg micro syndrome is caused by RAB18 deficiency or dysregulation. *Open Biol* 5: 150047
- Imai K, Hao F, Fujita N, Tsuji Y, Oe Y, Araki Y, Hamasaki M, Noda T, Yoshimori T (2016) Atg9A trafficking through the recycling endosomes is required for autophagosome formation. *J Cell Sci* 129: 3781–3791
- Kim JJ, Lipatova Z, Segev N (2016) TRAPP complexes in secretion and autophagy. *Front Cell Dev Biol* 4: 20
- Lamb CA, Nuhlen S, Judith D, Frith D, Snijders AP, Behrends C, Tooze SA (2016) TBC1D14 regulates autophagy via the TRAPP complex and ATG9 traffic. *EMBO J* 35: 281–301
- Li C, Luo X, Zhao S, Siu GKY, Liang Y, Chan HC, Satoh A, Yu SSB (2017) COPI–TRAPP II activates Rab18 and regulates its lipid droplet association. *EMBO J* 36: 441–457
- Lipatova Z, Majumdar U, Segev N (2016) Trs33-containing TRAPP IV: a novel autophagy-specific Ypt1 GEF. *Genetics* 204: 1117–1128
- Ozeki S, Cheng J, Tauchi-Sato K, Hatano N, Taniguchi H, Fujimoto T (2005) Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane. *J Cell Sci* 118: 2601–2611
- Scrivens PJ, Noueihed B, Shahrzad N, Hul S, Brunet S, Sacher M (2011) C4orf41 and TTC-15 are mammalian TRAPP components with a role at an early stage in ER-to-Golgi trafficking. *Mol Biol Cell* 22: 2083–2093
- Venditti R, Scanu T, Santoro M, Di Tullio G, Spaar A, Gaibisso R, Beznoussenko GV, Mironov AA, Mironov A Jr, Zelante L, Piemontese MR, Notarangelo A, Malhotra V, Vertel BM, Wilson C, De Matteis MA (2012) Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. *Science* 337: 1668–1672
- Wilfling F, Thiam AR, Olarte MJ, Wang J, Beck R, Gould TJ, Allgeyer ES, Pincet F, Bewersdorf J, Farese RV Jr, Walther TC (2014) Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. *Elife* 3: e01607
- Yamasaki A, Menon S, Yu S, Barrowman J, Meerloo T, Oorschot V, Klumperman J, Satoh A, Ferro-Novick S (2009) mTrs130 is a component of a mammalian TRAPP II complex, a Rab1 GEF that binds to COPI-coated vesicles. *Mol Biol Cell* 20: 4205–4215



License: This is an open access article under the terms of the Creative Commons Attribution 4.0 License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.