

Plasma membrane repairs by small GTPase Rab3a

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Lysosomes fuse with the plasma membrane to help repair membrane lesions, but how they are positioned close to these lesions is not fully understood. Now, Encarnaç o et al. (2016. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201511093>) demonstrate that the lysosomal GTPase Rab3a and its effectors orchestrate lysosome positioning and plasma membrane repair.

When the plasma membrane (PM) of eukaryotic cells is wounded by mechanical damage or by bacterial toxins, the wound is normally repaired within seconds by mechanisms triggered by the influx of extracellular Ca²⁺. A key mechanism for such repair is the fusion of lysosomes or late endosomes (for simplicity, referred to here as lysosomes) with the damaged area (Reddy et al., 2001). These lysosomes are located near the PM and are believed to fuse at multiple points around the lesion (Andrews et al., 2014). Like other cellular membrane-fusion events, such fusion requires specific SNARE proteins to pair with each other on the lysosome and the PM (Rao et al., 2004). Fusion is controlled by a Ca²⁺-sensing SNARE interacting protein in the lysosome membrane called synaptotagmin VII (Reddy et al., 2001).

Although lysosomes predominantly localize to the perinuclear region of most cells, a minor population of these degradative organelles can be found near the PM, where they can be rapidly mobilized to repair membrane wounds. Thus, mechanisms must exist for translocating lysosomes to the cell periphery and for anchoring them there for use in repairing the PM. The transport of lysosomes along microtubules, powered by members of the kinesin family of microtubule motors, has recently been shown to constitute an important mechanism for their long-range transport toward the PM (Korolchuk et al., 2011; Rosa-Ferreira and Munro, 2011; Pu et al., 2015; Raiborg et al., 2015). However, given the abundance of actin filaments underlying the PM, actin motors are also likely to play a key role in controlling the transport of PM-proximal lysosomes to the PM.

Vesicle transport, including vesicle attachment to motor proteins and their tethering to target membranes, is known to be controlled by small GTPases of the Rab family (Zhen and Stenmark, 2015). With regard to the positioning of lysosomes and related organelles, Rab7a promotes the attachment of Kinesin-1 to lysosomes and controls their transport to the cell periphery (Raiborg et al., 2015), whereas Rab27a and Rab27b promote the actin-dependent exocytosis of lysosome-related organelles, such as melanosomes and T cell cytotoxic granules (Fukuda, 2013). However, the involvement of the Rab GTPases

in lysosome-mediated PM repair has not been addressed. In this issue, Encarnaç o et al. screened siRNAs that target human Rab GTPases to identify Rabs involved in translocating lysosomes to the PM in response to the Ca²⁺ ionophore ionomycin and in the repair of PM holes caused by the bacterial toxin Streptolysin-O (Encarnaç o et al., 2016). One of the most prominent hits in this screen was Rab3a, a GTPase previously characterized mainly in the regulation of Ca²⁺-induced exocytosis of synaptic vesicles in neurons and of dense-core vesicles in endocrine cells (Gepfert et al., 1994; Johannes et al., 1994). Even though Rab3a is predominantly expressed in neurons and endocrine tissues, Encarnaç o et al. (2016) were able to detect its expression in HeLa cells and melanocytes. In these cells, they observed that a minor fraction of ectopically expressed Rab3a localizes to lysosomes, although most of the protein localizes to the Golgi complex.

How does Rab3a control lysosome positioning and PM repair? In general, Rab GTPases control vesicular traffic by recruiting effector proteins that bind exclusively to the GTP-bound, active form of the GTPase (Zhen and Stenmark, 2015). Previous studies have revealed several effectors for Rab3a, and when investigating the possible involvement of these in PM repair, Encarnaç o et al. (2016) observed that shRNA-mediated silencing of synaptotagmin-like protein 4-a (Slp4-a) causes the perinuclear clustering of lysosomes and the inhibition of PM repair. The authors also used coimmunoprecipitation experiments to identify a novel effector of Rab3a, nonmuscle myosin heavy chain IIA (NMHCIIA), an actin-based motor. As with Rab3a and Slp4-a, NMHCIIA is required to position lysosomes close to the PM. NMHCIIA has both contractile and structural (actin-bundling) roles, and Encarnaç o et al. (2016) speculate that it is the latter function that is relevant to PM repair. They propose that Rab3a recruits NMHCIIA to lysosomes to associate lysosomes with the cortical actin cytoskeleton; when a damage-induced Ca²⁺ influx occurs, the actin cytoskeleton reorganizes to allow lysosomes to be tethered to the PM, a process possibly mediated by Slp4-a. Because Slp4-a is not required for Rab3a and NMHCIIA to interact with each other, the two effectors probably function sequentially (Encarnaç o et al., 2016).

The identification of Rab3a and its effectors Slp4-a and NMHCIIA as regulators of lysosome positioning and PM repair represents a significant advance in our understanding of how lysosomes are mobilized to PM wounds. It is now becoming clear that Rab3a plays a key role in diverse exocytic processes, such as synaptic vesicle exocytosis, hormone release, and now PM sealing, and that the underlying mechanisms are variations

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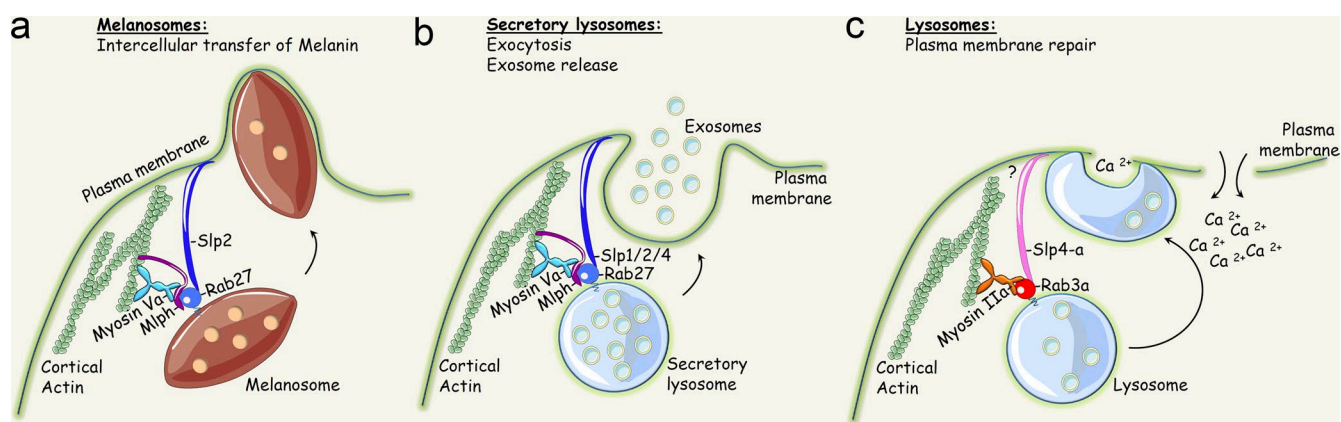


Figure 1. **Exocytosis of lysosomes and related organelles.** The schematic shows mechanisms for the exocytosis of melanosomes (a) and secretory lysosomes or multivesicular endosomes (b). (c) A speculative model for the function of Rab3a in lysosome-mediated PM repair.

on a theme in which the GTPase recruits a small set of effectors. The Rab3 proteins (including the a, b, c, and d isoforms) and the Rab27 proteins (the a and b isoforms) form their own branch of the Rab family tree (Diekmann et al., 2011), which has an evolutionarily conserved role in Ca^{2+} -regulated secretion. Fig. 1 summarizes the Rab-protein mediated mechanisms involved in the exocytosis of lysosomes and various related organelles.

The exocytosis of melanosomes, which mediates the transfer of the pigment melanin from melanocytes to keratinocytes, involves the translocation of melanosomes to the PM driven by the actin motor Myosin Va. The motor is attached to the melanosome via the Rab27a effector Mlph, an adaptor that connects to Rab27 in the melanosome membrane (Fig. 1; Strom et al., 2002). Another Rab27 effector, Slp2-a, is involved in tethering the melanosome to the PM (Kuroda and Fukuda, 2004; Fukuda, 2006). A similar mechanism is involved in the exocytosis of multivesicular endosomes, which leads to exosome release (Ostrowski et al., 2010), and to the exocytosis of lysosome-related organelles, such as T cell cytotoxic granules, which kill target cells (Stinchcombe et al., 2001). As with melanosome exocytosis, these processes involve Rab27, Mlph, Myosin Va, Slp2-a, and also Slp1 and Slp4-a (Fukuda, 2013). Given the new findings on Rab3a and its effectors, one can speculate about Rab3a functions that parallel those of Rab27. Even though Myosin IIA has primarily been assigned functions in tension-related cellular processes, such as cytokinesis and cell migration, this myosin has also been found to facilitate vesicle trafficking required for PM repair (Lin et al., 2012). It could therefore be involved in the actin-based motility of Rab3a-positive lysosomes close to the PM. Given the similarity of Slp4-a to Slp2-a and the involvement of Slp proteins in tethering lysosome-related organelles to the PM (Fukuda, 2006), it is also plausible that Slp4-a, bound to the lysosome via Rab3a, could mediate the tethering of the lysosome to the PM during PM repair (Fig. 1).

Further studies of Rab3a will reveal how these proteins function together in PM repair. It will be interesting to learn more about these molecular mechanisms and to compare them to other Rab3a-regulated exocytic processes, including synaptic vesicle release, dense-core vesicle exocytosis, and acrosome exocytosis (Geppert et al., 1994; Johannes et al., 1994; Yunes et al., 2000).

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References

- Andrews, N.W., P.E. Almeida, and M. Corrotte. 2014. Damage control: cellular mechanisms of plasma membrane repair. *Trends Cell Biol.* 24:734–742. <http://dx.doi.org/10.1016/j.tcb.2014.07.008>
- Diekmann, Y., E. Seixas, M. Gouw, F. Tavares-Cadete, M.C. Seabra, and J.B. Pereira-Leal. 2011. Thousands of rab GTPases for the cell biologist. *PLOS Comput. Biol.* 7:e1002217. <http://dx.doi.org/10.1371/journal.pcbi.1002217>
- Encarnaçao, M., L. Espada, C. Escrevente, D. Mateus, J. Ramalho, X. Michelet, I. Santarino, V.W. Hsu, M.B. Brenner, D. Barral, and O.V. Vieira. 2016. A Rab3a-dependent complex essential for lysosome positioning and plasma membrane repair. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201511093>
- Fukuda, M. 2006. Rab27 and its effectors in secretory granule exocytosis: a novel docking machinery composed of a Rab27-effector complex. *Biochem. Soc. Trans.* 34:691–695. <http://dx.doi.org/10.1042/BST0340691>
- Fukuda, M. 2013. Rab27 effectors, pleiotropic regulators in secretory pathways. *Traffic.* 14:949–963. <http://dx.doi.org/10.1111/tra.12083>
- Geppert, M., V.Y. Bolshakov, S.A. Siegelbaum, K. Takei, P. De Camilli, R.E. Hammer, and T.C. Südhof. 1994. The role of Rab3A in neurotransmitter release. *Nature.* 369:493–497. <http://dx.doi.org/10.1038/369493a0>
- Johannes, L., P.M. Lledo, M. Roa, J.D. Vincent, J.P. Henry, and F. Darchen. 1994. The GTPase Rab3a negatively controls calcium-dependent exocytosis in neuroendocrine cells. *EMBO J.* 13:2029–2037.
- Korolchuk, V.I., S. Saiki, M. Lichtenberg, F.H. Siddiqi, E.A. Roberts, S. Imarisio, L. Jahress, S. Sarkar, M. Futter, F.M. Menzies, et al. 2011. Lysosomal positioning coordinates cellular nutrient responses. *Nat. Cell Biol.* 13:453–460. <http://dx.doi.org/10.1038/ncb2204>
- Kuroda, T.S., and M. Fukuda. 2004. Rab27A-binding protein Slp2-a is required for peripheral melanosome distribution and elongated cell shape in melanocytes. *Nat. Cell Biol.* 6:1195–1203. <http://dx.doi.org/10.1038/ncb1197>
- Lin, P., H. Zhu, C. Cai, X. Wang, C. Cao, R. Xiao, Z. Pan, N. Weisleder, H. Takeshima, and J. Ma. 2012. Nonmuscle myosin IIA facilitates vesicle trafficking for MG53-mediated cell membrane repair. *FASEB J.* 26:1875–1883. <http://dx.doi.org/10.1096/fj.11-188599>
- Ostrowski, M., N.B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, C.F. Moita, K. Schauer, A.N. Hume, R.P. Freitas, et al. 2010. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* 12:19–30. <http://dx.doi.org/10.1038/ncb2000>
- Pu, J., C. Schindler, R. Jia, M. Jarnik, P. Backlund, and J.S. Bonifacino. 2015. BORC, a multisubunit complex that regulates lysosome positioning. *Dev. Cell.* 33:176–188. <http://dx.doi.org/10.1016/j.devcel.2015.02.011>

- Raiborg, C., E.M. Wenzel, N.M. Pedersen, H. Olsvik, K.O. Schink, S.W. Schultz, M. Vietri, V. Nisi, C. Bucci, A. Brech, et al. 2015. Repeated ER–endosome contacts promote endosome translocation and neurite outgrowth. *Nature*. 520:234–238. <http://dx.doi.org/10.1038/nature14359>
- Rao, S.K., C. Huynh, V. Proux-Gillardeaux, T. Galli, and N.W. Andrews. 2004. Identification of SNAREs involved in synaptotagmin VII-regulated lysosomal exocytosis. *J. Biol. Chem.* 279:20471–20479. <http://dx.doi.org/10.1074/jbc.M400798200>
- Reddy, A., E.V. Caler, and N.W. Andrews. 2001. Plasma membrane repair is mediated by Ca²⁺-regulated exocytosis of lysosomes. *Cell*. 106:157–169. [http://dx.doi.org/10.1016/S0092-8674\(01\)00421-4](http://dx.doi.org/10.1016/S0092-8674(01)00421-4)
- Rosa-Ferreira, C., and S. Munro. 2011. Arl8 and SKIP act together to link lysosomes to kinesin-1. *Dev. Cell*. 21:1171–1178. <http://dx.doi.org/10.1016/j.devcel.2011.10.007>
- Stinchcombe, J.C., D.C. Barral, E.H. Mules, S. Booth, A.N. Hume, L.M. Machesky, M.C. Seabra, and G.M. Griffiths. 2001. Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J. Cell Biol.* 152:825–834. <http://dx.doi.org/10.1083/jcb.152.4.825>
- Strom, M., A.N. Hume, A.K. Tarafder, E. Barkagianni, and M.C. Seabra. 2002. A family of Rab27-binding proteins. Melanophilin links Rab27a and myosin Va function in melanosome transport. *J. Biol. Chem.* 277:25423–25430. <http://dx.doi.org/10.1074/jbc.M202574200>
- Yunes, R., M. Michaut, C. Tomes, and L.S. Mayorga. 2000. Rab3A triggers the acrosome reaction in permeabilized human spermatozoa. *Biol. Reprod.* 62:1084–1089. <http://dx.doi.org/10.1095/biolreprod62.4.1084>
- Zhen, Y., and H. Stenmark. 2015. Cellular functions of Rab GTPases at a glance. *J. Cell Sci.* 128:3171–3176. <http://dx.doi.org/10.1242/jcs.166074>