Beyond the SNARE: Munc18-1 chaperones α-synuclein

Mugdha Deshpande and Avital A. Rodal

Department of Biology, Brandeis University, Waltham, MA 02453

Early infantile epileptic encephalopathy (EIEE)–associated mutations in *MUNC18-1* cause Munc18-1 misfolding and cellular aggregation. In this issue, Chai et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201512016) find that Munc18-1 is a molecular chaperone for α-synuclein and that aggregated Munc18-1 EIEE-causing mutants promote α-synuclein aggregation.

Early infantile epileptic encephalopathy (EIEE), also known as Ohtahara syndrome, is a debilitating neurological disorder that results in early-onset tonic seizures and severe intellectual disability. Heterozygous mutations in *MUNC18-1* (also known as syntaxin binding protein 1 [*STXBP1*]) are linked to EIEE (Saitsu et al., 2008; Stamberger et al., 2016). Munc18-1 was first identified as an essential component of the synaptic vesicle fusion machinery and binds to the SNARE receptor Syntaxin-1A (Hata et al., 1993). Munc18-1 plays an important role in regulating neuronal exocytosis by acting as a molecular chaperone for Syntaxin-1A and regulating its levels at the plasma membrane and has additional functions in vesicle fusion (Han et al., 2011; Ma et al., 2015; Shen et al., 2015). Although the genetic link between Munc18-1 and EIEE has been established through multiple studies, it remains unclear how loss of Munc18-1 leads to EIEE. One EIEE-associated Munc18-1 mutation (C180Y) is located in the hydrophobic core of the protein and results in decreased thermostability (Saitsu et al., 2008; Martin et al., 2014). Although Munc18-1^{C180Y} retains the ability to bind Syntaxin-1A, it has a tendency to form intracellular aggregates, which are targeted for proteosomal degradation (Martin et al., 2014). Therefore, one model to explain EIEE-associated haploinsufficiency is that Munc18-1^{C180Y} mutants draw wild-type Munc18-1 proteins into aggregates, thereby lowering the levels of available functional molecules. In this issue, Chai et al. directly tested this model by analyzing aggregate formation in the presence of Munc18-1^{C180Y} mutants.

Using single-molecule fluorescence spectroscopy in a cell-free system, Chai et al. (2016) found that mutant Munc18-1 coaggregated with wild-type Munc18-1 in vitro. The researchers tested the ability of Munc18-1^{C180Y} to recruit new monomers, and thus seed larger aggregates, by using Munc18-1^{C180Y} tagged with two different fluorophores. These experiments showed that small aggregates of the mutant protein could seed formation of larger fibrils composed not only of the mutant Munc18-1 but also of the wild-type protein. Coaggregation with wild-type protein also occurred in vivo, when the authors expressed Munc18-1^{C180Y} in PC12 pheochromocytoma cells or in rat hippocampal neurons. Several other EIEE-associated Munc18-1 mutants exhibited a similar ability to cause wild-type Munc18-1 aggregation in cells. Thus, the authors conclude that EIEE mutants are likely to act dominantly by sequestering wild-type Munc18-1 into aggregates (Fig. 1 A).

A surprising twist came when Chai et al. (2016) noticed Lewy body–like structures in Munc18-1^{C180Y}–expressing cells. Lewy bodies are fibrillar intracellular inclusions that are hallmarks of Parkinson's disease and Lewy body dementia. The primary component of Lewy bodies is α-synuclein, a presynaptic protein highly expressed in the brain (Burré, 2015). The researchers followed up on this observation by showing that α-synuclein coaggregated with Munc18-1^{C180Y} oligomers by single-molecule fluorescence spectroscopy in the cell-free system. In PC12 cells and in hippocampal neurons, expression of Munc18-1^{C180Y} and other EIEE-linked mutants resulted in recruitment of α-synuclein into Lewy body–like aggregates. Conversely, Parkinson's disease–associated aggregation-prone mutations in α-synuclein drew wild-type Munc18-1 into aggregates (Fig. 1 B). Finally, Chai et al. (2016) showed that endogenous α-synuclein coimmunoprecipitated with endogenous Munc18-1. Together, these results point to the interesting new hypothesis that Munc18-1 serves as a chaperone for α-synuclein, in addition to its role in SNARE regulation. A molecular chaperone facilitates proper folding, assembly, and disassembly of its target proteins and prevents aggregation. In support of a role for endogenous Munc18-1 in chaperoning α-synuclein, Chai et al. (2016) showed that loss of Munc18-1 leads to increased α-synuclein aggregation, in both neurosecretory cells and in hippocampal neurons (Fig. 1 C). This α-synuclein aggregation phenotype could be rescued by reintroducing Munc18-1 in a dose-dependent manner. Thus, the ability of endogenous Munc18-1 to bind α-synuclein and to control its capacity to aggregate suggests that it has a physiological chaperone-like function for α-synuclein.

What role does the Munc18-1–α-synuclein interaction play in a cell, and how might this impinge on the pathological roles of α-synuclein in neurological disease? α-Synuclein binds directly to membranes and has several proposed functions in normal membrane trafficking, including serving as a chaperone for SNARE assembly via an interaction with the SNARE synaptobrevin-2 (Burré et al., 2010), as well as additional functions in diverse other processes, including membrane remodeling, lipid metabolism, neurotransmitter synthesis and transport, and synaptic vesicle mobilization (Burré, 2015). In pathological states

Correspondence to Avital A. Rodal: arodal@brandeis.edu

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 214 No. 6 641–643
www.jcb.org/cgi/doi/10.1083/jcb.201608060

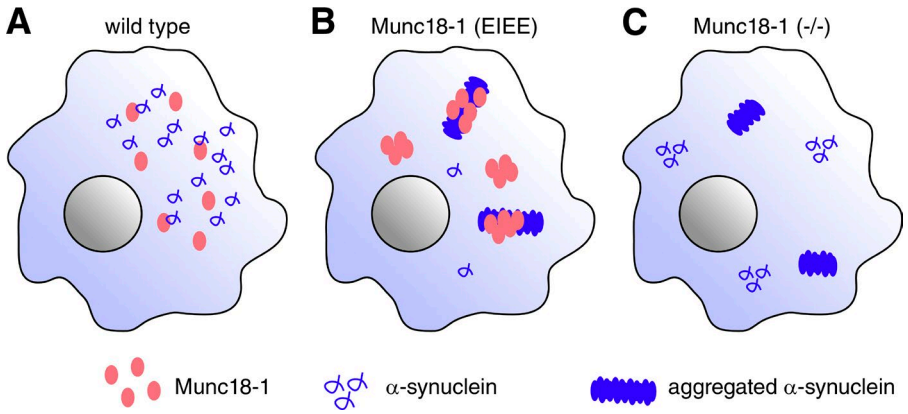

Figure 1. **Munc18-1 acts as a chaperone for α-synuclein.** (A) Healthy cells. (B) EIEE-linked mutants in Munc18-1 cause haploinsufficiency by seeding aggregation of wild-type Munc18-1 and causing aggregation of α-synuclein. (C) α-Synuclein aggregates in the absence of Munc18-1.

such as Parkinson's disease, α-synuclein misfolds and assembles into aggregates that cause neurotoxicity and can be transmitted in a prion-like form from neuron to neuron (Hasegawa et al., 2016). One hypothesis is that Munc18-1 indirectly prevents α-synuclein aggregation by chaperoning SNAREs. Membrane binding by α-synuclein suppresses its aggregation (Zhu and Fink, 2003; Burré et al., 2015), and reduction of SNAREs at the membrane in Munc18-1 mutants may in turn affect membrane α-synuclein levels, resulting in its aggregation. However, Chai et al. (2016)'s finding that Munc18-1 and α-synuclein coaggregate in a cell-free system (where cellular and biochemical interactions with SNAREs are not relevant) suggests that Munc18-1 may instead act directly on α-synuclein. It will be interesting to test how Munc18-1–mediated chaperoning affects the ordered oligomerization of α-synuclein on the membrane into aggregation-protective, physiologically functional conformations and to investigate if this chaperone activity plays a role in Parkinson's disease and other related synucleinopathies.

Another interesting point to consider is how and why so many layers of chaperone activity are incorporated into the synaptic vesicle fusion apparatus. This protein machinery must undergo an enormous number of energetically demanding assembly and disassembly events during its lifetime ($t_{1/2}$ of ∼2–5 d [Cohen et al., 2013]), leaving neurons extremely sensitive to accumulation of misfolded material. New findings by Chai et al. (2016) indicate that Munc18-1 is a chaperone for α-synuclein, in addition to its previously defined activity on Syntaxin-1A and at subsequent steps of SNARE-mediated fusion. α-Synuclein itself chaperones SNARE assembly via an interaction with the SNARE synaptobrevin-2 (Burré et al., 2015). Finally, the synaptic vesicle–associated protein CSPα functions with several cofactors, including Hsc70, to chaperone the SNARE synaptosomal-associated protein of 25 Kd (SNAP-25; Sharma et al., 2011). Interestingly, α-synuclein overexpression compensates for the severe neurodegenerative phenotypes arising from loss of CSPα without rescuing altered SNAP-25 levels (Chandra et al., 2005), suggesting that although the specific clients of each chaperone are nonredundant, driving proper protein conformation at any step of the SNARE pathway is sufficient to support neuronal health in vivo. Munc18-1–mediated chaperoning of α-synuclein may provide another mechanism for ensuring a continuous supply of functional SNARE machinery proteins.

How might aggregation of Munc18-1 cause EIEE? A first hint came from the finding that several EIEE-causing mutations are heterozygous intragenic or whole-gene deletions, suggesting that haploinsufficiency for normal Munc18-1 function accounts for the disease (Saitsu et al., 2008; Stamberger et al., 2016). This hypothesis is also consistent with Chai et al. (2016)'s findings that aggregation-prone Munc18-1 mutants sequester the wild-type copy of Munc18-1. Human cells may be even more sensitive to partial loss of *MUNC18-1* because *MUNC18-1* heterozygous human induced pluripotent stem cell–derived neurons exhibit significant defects in synaptic strength (Patzke et al., 2015), whereas heterozygous mouse neurons display more subtle phenotypes (Toonen et al., 2006). Further insight into EIEE might therefore be gleaned from loss-of-function animal models of the disease. *Munc18-1*–null mutant mice exhibit normal brain development despite lacking synaptic transmission. However, unlike many other synaptic transmission mutants, these mice show widespread cell-autonomous neuronal death and their spinal cords feature hallmarks of neurodegeneration, including tau and ubiquitin-positive inclusions (Law et al., 2016). These neurodegenerative features may extend to EIEE mutants as well; Chai et al. (2016) found that cultured neurons expressing Munc18-1^{C180Y} develop pyknosis (nuclei with condensed chromatin), indicating that they are undergoing cell death. Although it is clear that Munc18-1 EIEE mutants have defects in synaptic transmission (Martin et al., 2014; Shen et al., 2015), it will be important to further discern if neurodegeneration also contributes to the pathology of EIEE in humans and in animal models. Interestingly, overexpression of Syntaxin-1A or Syntaxin-1B partially rescues neuronal viability defects in *MUNC18-1* mutant neurons in culture, suggesting that neurodegeneration is at least in part mediated via the SNARE-dependent function of Munc18-1 (Vardar et al., 2016). In the future, it will be of great interest to test in vivo how neurodegeneration depends on the role of Munc18-1 in vesicle fusion or in chaperoning SNARES, α-synuclein, or other Munc18-1 targets.

Acknowledgments

The authors thank Steve Del Signore and Rylie Walsh for helpful comments.

The authors are supported by a Blazeman Foundation for ALS research postdoctoral fellowship (M. Deshpande) and National Institutes of Health grant DP2 NS60947 (A.A. Rodal).

The authors declare no competing financial interests.

Submitted: 18 August 2016
Accepted: 22 August 2016

References

Burré, J. 2015. The synaptic function of α-synuclein. *J. Parkinsons Dis.* 5:699–713. http://dx.doi.org/10.3233/JPD-150642

Burré, J., M. Sharma, T. Tsetsenis, V. Buchman, M.R. Etherton, and T.C. Südhof. 2010. α-Synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science.* 329:1663–1667. http://dx.doi.org/10.1126/science.1195227

Burré, J., M. Sharma, and T.C. Südhof. 2015. Definition of a molecular pathway mediating α-synuclein neurotoxicity. *J. Neurosci.* 35:5221–5232. http://dx.doi.org/10.1523/JNEUROSCI.4650-14.2015

Chai, Y.J., E. Sierecki, V.M. Tomatis, R.S. Gormal, N. Giles, I.C. Morrow, D. Xia, J. Götz, R.G. Parton, B.M. Collins, et al. 2016. Munc18-1 is a molecular chaperone for α-synuclein controlling its self-replicating aggregation. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201512016

Chandra, S., G. Gallardo, R. Fernández-Chacón, O.M. Schlüter, and T.C. Südhof. 2005. α-Synuclein cooperates with CSPα in preventing neurodegeneration. *Cell.* 123:383–396. http://dx.doi.org/10.1016/j.cell.2005.09.028

Cohen, L.D., R. Zuchman, O. Sorokina, A. Müller, D.C. Dieterich, J.D. Armstrong, T. Ziv, and N.E. Ziv. 2013. Metabolic turnover of synaptic proteins: Kinetics, interdependencies and implications for synaptic maintenance. *PLoS One.* 8:e63191. http://dx.doi.org/10.1371/journal.pone.0063191

Han, G.A., N.T. Malintan, N.M. Saw, L. Li, L. Han, F.A. Meunier, B.M. Collins, and S. Sugita. 2011. Munc18-1 domain-1 controls vesicle docking and secretion by interacting with syntaxin-1 and chaperoning it to the plasma membrane. *Mol. Biol. Cell.* 22:4134–4149. http://dx.doi.org/10.1091/mbc.E11-02-0135

Hasegawa, M., T. Nonaka, and M. Masuda-Suzukake. 2016. α-Synuclein: Experimental pathology. *Cold Spring Harb. Perspect. Med.* a024273. http://dx.doi.org/10.1101/cshperspect.a024273

Hata, Y., C.A. Slaughter, and T.C. Südhof. 1993. Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. *Nature.* 366:347–351. http://dx.doi.org/10.1038/366347a0

Law, C., M. Schaan Profes, M. Levesque, J.A. Kaltschmidt, M. Verhage, and A. Kania. 2016. Normal molecular specification and neurodegenerative disease-like death of spinal neurons lacking the SNARE-associated synaptic protein Munc18-1. *J. Neurosci.* 36:561–576. http://dx.doi.org/10.1523/JNEUROSCI.1964-15.2016

Ma, L., A.A. Rebane, G. Yang, Z. Xi, Y. Kang, Y. Gao, and Y. Zhang. 2015. Munc18-1-regulated stage-wise SNARE assembly underlying synaptic exocytosis. *eLife.* e09580. http://dx.doi.org/10.7554/eLife.09580

Martin, S., A. Papadopulos, V.M. Tomatis, E. Sierecki, N.T. Malintan, R.S. Gormal, N. Giles, W.A. Johnston, K. Alexandrov, Y. Gambin, et al. 2014. Increased polyubiquitination and proteasomal degradation of a Munc18-1 disease-linked mutant causes temperature-sensitive defect in exocytosis. *Cell Reports.* 9:206–218. http://dx.doi.org/10.1016/j.celrep.2014.08.059

Patzke, C., Y. Han, J. Covy, F. Yi, S. Maxeiner, M. Wernig, and T.C. Südhof. 2015. Analysis of conditional heterozygous STXBP1 mutations in human neurons. *J. Clin. Invest.* 125:3560–3571. http://dx.doi.org/10.1172/JCI78612

Saitsu, H., M. Kato, T. Mizuguchi, K. Hamada, H. Osaka, J. Tohyama, K. Uruno, S. Kumada, K. Nishiyama, A. Nishimura, et al. 2008. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat. Genet.* 40:782–788. http://dx.doi.org/10.1038/ng.150

Sharma, M., J. Burré, and T.C. Südhof. 2011. CSPα promotes SNARE-complex assembly by chaperoning SNAP-25 during synaptic activity. *Nat. Cell Biol.* 13:30–39. http://dx.doi.org/10.1038/ncb2131

Shen, C., S.S. Rathore, H. Yu, D.R. Gulbranson, R. Hua, C. Zhang, N.E. Schoppa, and J. Shen. 2015. The *trans*-SNARE-regulating function of Munc18-1 is essential to synaptic exocytosis. *Nat. Commun.* 6:8852. http://dx.doi.org/10.1038/ncomms9852

Stamberger, H., M. Nikanorova, M.H. Willemsen, P. Accorsi, M. Angriman, H. Baier, I. Benkel-Herrenbrueck, V. Benoit, M. Budetta, A. Caliebe, et al. 2016. *STXBP1* encephalopathy: A neurodevelopmental disorder including epilepsy. *Neurology.* 86:954–962. http://dx.doi.org/10.1212/WNL.0000000000002457

Toonen, R.F., K. Wierda, M.S. Sons, H. de Wit, L.N. Cornelisse, A. Brussaard, J.J. Plomp, and M. Verhage. 2006. Munc18-1 expression levels control synapse recovery by regulating readily releasable pool size. *Proc. Natl. Acad. Sci. USA.* 103:18332–18337. http://dx.doi.org/10.1073/pnas.0608507103

Vardar, G., S. Chang, M. Arancillo, Y.J. Wu, T. Trimbuch, and C. Rosenmund. 2016. Distinct functions of syntaxin-1 in neuronal maintenance, synaptic vesicle docking, and fusion in mouse neurons. *J. Neurosci.* 36:7911–7924. http://dx.doi.org/10.1523/JNEUROSCI.1314-16.2016

Zhu, M., and A.L. Fink. 2003. Lipid binding inhibits α-synuclein fibril formation. *J. Biol. Chem.* 278:16873–16877. http://dx.doi.org/10.1074/jbc.M210136200