Published in final edited form:
Knorr, R. L., Mizushima, N., & Dimova, R. (2017). Fusion and scission of membranes: ubiquitous topological transformations in cells. *Traffic*, 18(11), 758-761. doi:10.1111/tra.12509.

Fusion and scission of membranes: ubiquitous topological transformations in cells

Roland L. Knorr[1*], Noboru Mizushima[2], Rumiana Dimova[1]
[1]Max Planck Institute of Colloids and Interfaces; Science Park Golm; Potsdam, Germany
[2]Graduate School and Faculty of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

Synopsis: Remodeling of lipid membranes involves gradual bending as well as abrupt events: membrane fusion and membrane scission. Such discontinuous changes alter the number of individual (separate) membranes or the number of (torus-like) holes within the membrane structure: the topology of the structure changes. Here, we review cell membrane remodeling from a topological viewpoint, highlight the large number of topological changes during autophagy and link the two 2016 Nobel prizes honoring autophagy and topology.

Fusion and scission of membranes: ubiquitous topological transformations in cells

Running title: Fusion and scission of membranes

Roland L. Knorr[1*], Noboru Mizushima[2], Rumiana Dimova[1]

[1]Max Planck Institute of Colloids and Interfaces; Science Park Golm; Potsdam, Germany

[2]Graduate School and Faculty of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

* knorr@mpikg.mpg.de, Tel: +49 331 567 9668; Fax: +49 331 567 9612

Keywords: membrane, lipid, fusion, fission, scission, bending, topology, autophagy, ATG, two-dimensional, surface

Synopsis: Remodeling of lipid membranes involves gradual bending as well as abrupt events: membrane fusion and membrane scission. Such discontinuous changes alter the number of individual (separate) membranes or the number of (torus-like) holes within the membrane structure: the topology of the structure changes. Here, we review cell membrane remodeling from a topological viewpoint, highlight the large number of topological changes during autophagy and link the two 2016 Nobel prizes honoring autophagy and topology.

Abstract: 2016 Nobel Prizes were awarded to Yoshinori Ohsumi for autophagy and to David Thouless, Duncan Haldane and Michael Kosterlitz for topological transitions. Both of these phenomena are intrinsically related when it comes to membranes. Here, we give a brief account on topological transformations of lipid membranes, commonly known as membrane fusion and membrane scission, and introduce the underlying topological invariant, the genus. The genus of a shape is a useful concept to distinguish unambiguously the processes of membrane fusion/scission and offers a simple method to describe complex, cellular membrane structures, such as fenestrated cristae. We distinguish and highlight the connection between topological transformations of lipid membranes and the recent awards, and point out the extraordinarily large number of topological changes during autophagy.

For those who contribute to the "greatest benefit on mankind"[1] the Nobel prizes are awarded annually. Today, six disciplines are considered: physics, chemistry, physiology or medicine, literature, peace and economics. Given the diversity of the fields these categories span, it is unusual that a scientific topic is highlighted by more than one prize in the same year. Was 2016 an outlier? Prizes were awarded for groundbreaking discoveries in the area of autophagy to Yoshinori Ohsumi and for topological transitions in two-dimensional materials to David Thouless, Duncan Haldane and Michael Kosterlitz.

Macroautophagy (autophagy herein) is a very complex intracellular pathway, leading to the membrane-bound degradation of cytoplasmic material. In 1993 Yoshinori Ohsumi published the first list of 15 yeast *atg* mutants that are defective in autophagic degradation in the vacuole under starvation conditions.[2] This laid the foundation for a new, highly active scientific field.[3] Autophagy is essential for healthy development and impaired autophagy could contribute to various diseases such as cancer and neurodegenerative disorders.[4] During autophagy, short-lived organelles called autophagosomes are formed de novo in the cytoplasm. The development and degradation of these organelles involves very complex processes inside cells and imposes many shape alterations of the autophagic lipid membranes.

Topology is concerned with the features of structures that change abruptly. For example, if we consider the division of one object into two, the number of objects is either one or two but never attains an intermediate value. Thus, alterations in topology occur only in discrete steps and they are described by integers, see Fig. 1. The topological perspective of the work of David Thouless, Duncan Haldane and Michael Kosterlitz in the 1970s and 1980s resulted in an entirely new understanding of phase transitions of condensed matter. Thanks to their theories, we now relate a number of mysterious phenomena to changes in topology: the coupling of spin vortices of two-dimensional magnets; the stepwise changes in electrical conductance of thin conducting layers; and the different effects of spin on chains of atomic magnets.[5-7] Their research has paved a new frontier in condensed matter physics in recent years and raised hope that topological materials will be useful for new electronics and superconductors.

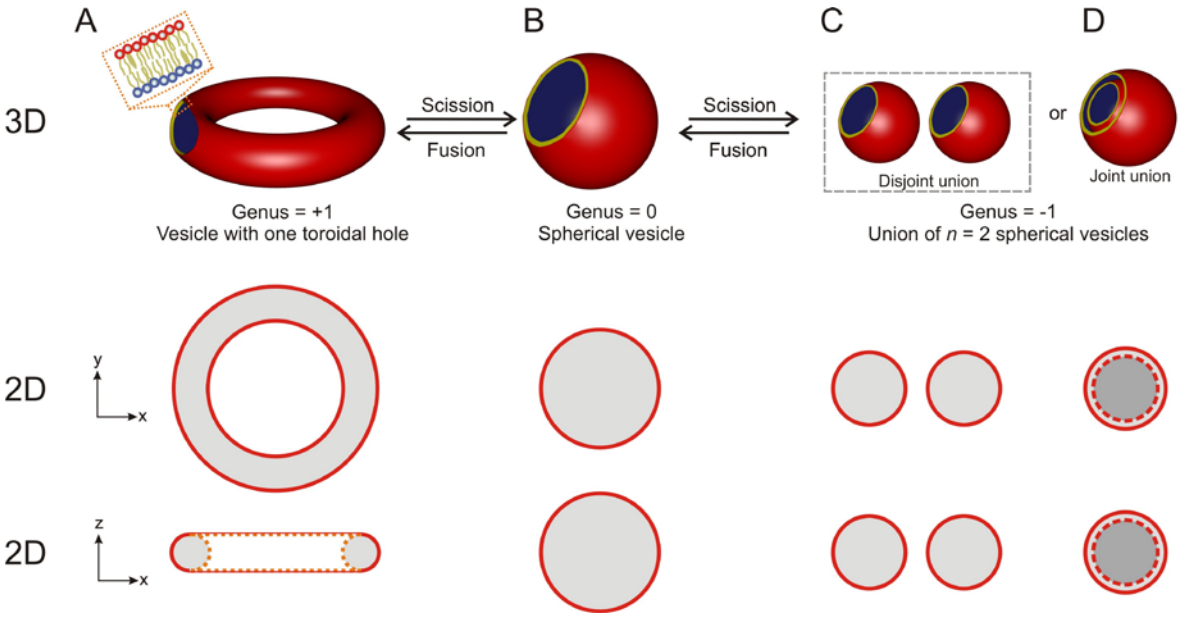

Fig 1. Membrane scission and membrane fusion change the topology of lipid membranes. Whereas scission decreases the genus of the surface, fusion increases the genus. To make the number of constituent lipid bilayers visible, 3-dimensional shapes are displayed with a side cut of the surface, see inset in (A), and 2-dimensional sections are included for two perspectives. (A-C) Vesicular structures built from a single membrane. (D) When one membrane encloses a second one they form a joint union or double-membrane structure. Such morphologies form by cup-shaped intermediates, Fig. 3 or inward invagination. Examples for joint unions in the cell are autophagosomes,

forespore membranes or “multi”-vesicular bodies with just a single inner vesicle. The genus of such morphologies is equivalent to a disjoint union of two single vesicles (C). 2-dimensional views show membranes in red. Dashed lines are membranes visible only in sections, dotted lines are toroidal holes, and differences in grey scale refer to separated lumens. Changes in membrane morphology required to transition between the different states are not shown for clarity.

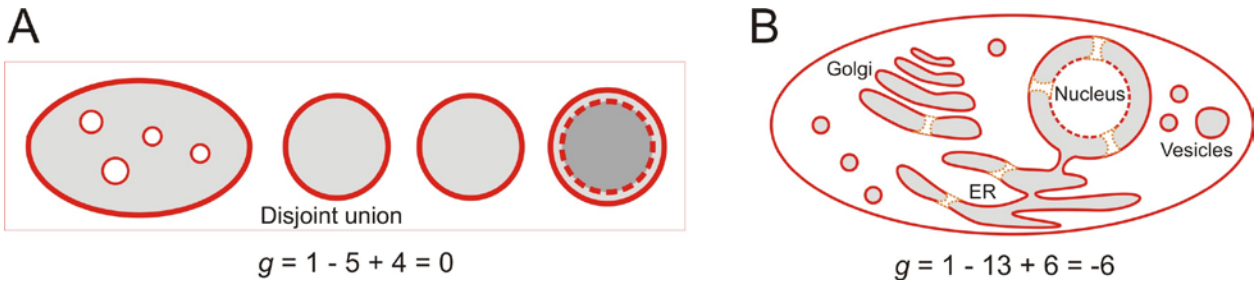

Fig 2. The genus of multivesicular membrane assemblies. The genus is calculated according to $g = 1 - n + h$, where n and h are the number of vesicular membranes and holes, respectively. (A) Disjoint union of 5 vesicles perforated by 4 toroidal holes. (B) Eukaryotic cell can be considered as union of multiple membranes with numerous holes. The 2-dimensional views are drawn as in Fig.1.

Lipid and biological membranes are fluids. Because of its small thickness, membranes can be considered as quasi-two-dimensional materials which form closed vesicular structures. These structures can also undergo changes of topological nature, Fig 1. The parameter used to quantify such changes is an integer topological invariant, the genus g. The genus of a connected, orientable surface (such as lipid membranes) is equal to the number of holes h (or handles). Holes in this context refers to structures maintaining a continuous membrane surface without disrupting the structure of the lipid bilayer. Thus, for a vesicle with a single toroidal hole, $g = 1$, Fig 1A. The genus of a spherical vesicle is $g = 0$, Fig 1B. Transforming one shape into the other shape alters g and thus, is a change in the membrane topology.

When the genus is increased, membrane fusion occurs, when the genus is decreased the process is referred to as membrane scission (or membrane fission), Fig. 1A, B. Toroidal holes can develop and vanish inside cells in the form of nuclear pores, by changing the connectivity of the network formed by the endoplasmic reticulum or fenestration of Golgi-cristae. Much more prominent however, are two other forms of topological transformations: the generation of lipid vesicles from parental membranes by membrane scission, such as during anterograde or retrograde transport. Later, such lipid vesicles undertake a second topological transformation that is membrane fusion with target membranes, Fig. 1B, C.

A single lipid vesicle with $g = 0$ undergoing membrane scission forms two vesicles, each with $g = 0$ again. This seems to disagree with the definition of a topological change, requiring a change of the genus. However, *all* resulting surfaces, i.e. both vesicles, need to be considered for the calculation of the genus according to $g = 1 - n + h$, where n is the number of membrane surfaces (in this case two vesicles) and h the number of holes. Thus, $g = -1$ for two vesicles. Note, for the calculation of the genus, the position of the vesicles relative to each other is irrelevant: a system with two vesicles next to each other is has the same genus as that of a system where one enclosing the other, Fig. 1 C, D. Complex assemblies of membranes, including

holes, can be characterized by the genus as well, Fig. 2.

In such a topological perspective, the eukaryotic cell can be understood as a union of many unconnected membranes (organelles and vesicles), enclosed by the plasma membrane. However, as some organelles, such as the network of the endoplasmic reticulum, the Golgi apparatus or the nuclear membrane, contain a large number of holes, the genus of a cell is not that easy to determine, Fig. 2B. Not even the sign of the genus can be estimated, since it is not known at present whether the number of organelles and vesicles is greater than the number of holes, or vice versa. For example, recent work demonstrated that sheets of the endoplasmic reticulum are highly fenestrated.[8] Thus, these structures are better described as densely packed tubular networks instead of sheets, suggesting a much higher *g* than anticipated previously. Moreover, it seems reasonable that the genus of a cell depends on a large variety of parameters, for example the cell type (secretory cells for instance contain a lot of vesicles), developmental status or cell cycle. New whole-cell high resolution imaging techniques, such as scanning electron microscopy combined with focused ion beam milling or a combination of various superresolution techniques, are promising tools with the perspective in the near future to describe entire cells in geometric terms.[8, 9]

Topological transformations in cells take place during autophagy, in all vesicular transport pathways, as well as during viral infections and release, neurotransmission and hormone release, in the endolysosomal system, cell division or dynamics of organelles such as mitochondria. Moreover, applications such as liposomal drug delivery or production of monoclonal antibodies (cell-cell fusion) depend on changes in membrane topology.[10-13] In cells, proteins or complexes such as dynamins, COPs, ESCRTs or SNAREs are required to overcome energy barriers associated with topological changes.[10] In addition local changes in pH, as occurring in endosomes, can support membrane fusion as is the case for the influenza HA protein or pH sensitive liposomal drugs.

An extraordinarily large number of changes in the membrane shape characterize the process of autophagy. Up to five steps can be distinguished: two early and two late topological transformations, with a morphological transformation in between, Fig. 3. The two early transformations include a membrane scission event (step 1) and a membrane fusion event (step 2). Both are fundamental for the formation of preautophagosomal membranes. Membrane lipids are provided in the form of vesicles from cellular organelles via membrane scission (In yeast, Atg9 vesicles are fissioned of the Golgi-apparatus most likely.[14]). The assembly of the so-called isolation membranes or phagophores occurs by fusion of such vesicles. The initial shapes of phagophores are rather flat membrane sheets (with continuous curved edges). Their expansion during the onset of autophagy increases the length of their strongly curved edge and thus the energetic costs of keeping the phagophore flat. The removal of the unfavorable edge contributes a significant amount of energy to allow curving of the sheet-like structure into a cup-like shape (step 3), and as a result, the cytosolic cargo is captured, Fig. 3.[15] Phagophore bending is followed by two late topological transformations which include one membrane scission and another membrane fusion step.[16] First, the single continuous membrane of the phagophore is split into two membranes: the outer and the inner autophagosomal vesicle (step 4). This scission step is followed by fusion of the outer autophagosomal vesicle with lysosomes, Fig.3, step 5. The latter initiates the degradation of the inner autophagosomal membrane together with the captured

material. Changing the sequence of the topological transformations, for example step 5 occurring before step 4, can induce reopening/flattening of phagophores into cup-shaped organelles (reversing step 3),[17] most likely aborting autophagy with the corresponding physiological consequences. This suggests that the order of late topological changes is conserved.

Similarly to non-autophagic membrane fusion and scission events in cells, energy barriers associated with topological changes during autophagy most probably proceed only by active processes. Thus, it is reasonable that known fusion and scission proteins or complexes such as SNAREs or ESCRTs intercalate with ATGs to regulate and drive these processes.

Conclusion

A lipid membrane can be considered as a two-dimensional material. When environmental conditions are altered, topological changes of the membrane surface can be induced, altering *g* in integer steps: half a hole cannot be formed in a membrane. As discussed, such changes represent modifications in the *geometry of the surface*. In contrast, David Thouless, Duncan Haldane and Michael Kosterlitz focused on *physical properties of material*.[5-7] They studied transitions in two-dimensional layers in response to a variation of external conditions. Spin vortices of two-dimensional magnets are either coupled or uncoupled for example, but not half-coupled. The coupling can be defined by integer numbers and is now considered as topological phase transition. Thus, the link between the two 2016 Nobel prizes honoring autophagy and topological phase transitions is that in both cases topological invariants change in discrete, integer steps.

With this comment, we highlight that autophagic membranes undergo a large number of topological transformations, which has not been previously considered. Understanding autophagy in the context of the shape transformations of the two-dimensional, fluid membrane surface will help to unravel the links between topological changes and the underlying biochemical networks formed by ATGs (and additional interaction partners) in detail. 24 years after the discovery of the first ATGs by Yoshinori Ohsumi, many secrets of the pathway await to be revealed, and applying this knowledge may help us with the treatment of a wide variety of complex and life-threatening diseases.

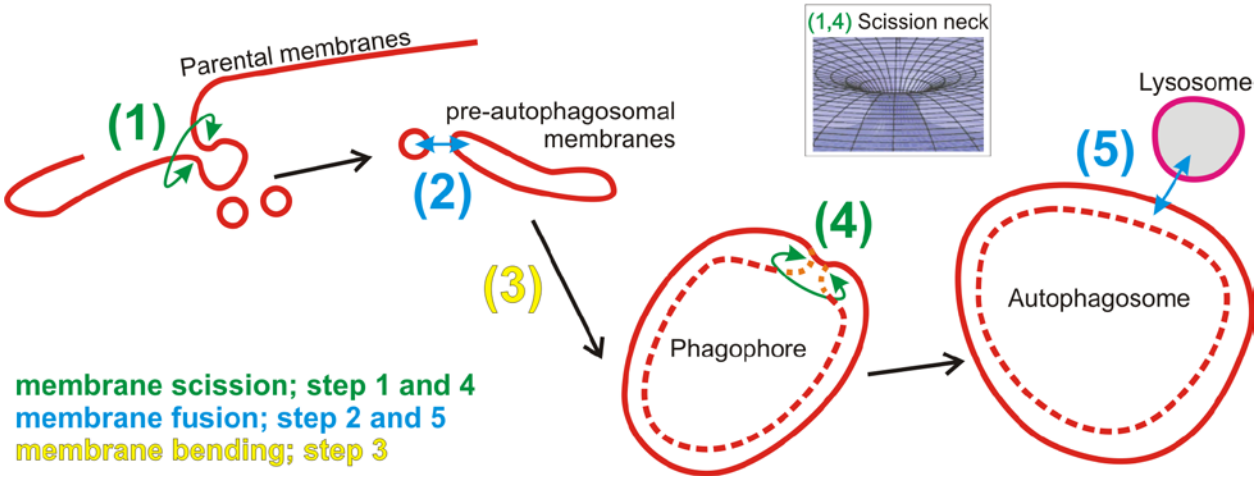

Fig 3: Shape transformations of lipid membranes during autophagy. Autophagy is characterized by four topological transformations (two early ones and two late ones), with one morphological change in between. (Step 1): Membrane vesicles are derived from parental membranes such as the endoplasmic reticulum (ER) and the Golgi-apparatus by numerous membrane scission events. (Step 2): Such small vesicles merge via membrane fusion and form flat, preautophagosomal membranes, whose initial shape is equivalent to membrane sheets or cristae. The phagophores expand by a continuous membrane supply. (Step 3): Their strongly bent, energetically unfavorable rim is reduced by bending of the sheet-like phagophore. (Step 4): For the formation of the autophagosome, a transient organelle bound by two membranes, a second membrane scission is required to separate the outer from the inner autophagosomal vesicle. (Step 5): Autophagosomal degradation is initiated by membrane fusion of the outer vesicle with lysosome. Inset: 3D view of the membrane scission neck.[16] Membrane scission necks are indicated by green arrows, membrane fusion events by blue arrows. Note that bending is involved in all topological transformations.

Funding: This work is part of the MaxSynBio consortium which is jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgement: We thank R. Lipowsky for stimulating discussions, R. Genz for help with 3D images, S. Laser and Y. Fischer supported the abstract figure.

References

1. Nobel A. Last will. w[3]nobelprizeorg/alfred_nobel/will/.
2. Tsukada M, Ohsumi Y. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. Febs Lett 1993;333(1-2):169-174.
3. Mizushima N, Yoshimori T, Ohsumi Y. The Role of Atg Proteins in Autophagosome Formation. Annu Rev Cell Dev Bi 2011;27:107-132.
4. Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. N Engl J Med 2013;368(19):1845-1846.
5. Haldane FDM. Nonlinear Field Theory of Large-Spin Heisenberg Antiferromagnets: Semiclassically Quantized Solitons of the One-Dimensional Easy-Axis Néel State. Phys Rev Lett 1983;50(15).
6. Kosterlitz JM, Thouless DJ. Long-Range Order and Metastability in 2-Dimensional Solids and Superfluids. J Phys Part C Solid 1972;5(11):L124-+.
7. Kosterlitz JM, Thouless DJ. Ordering, Metastability and Phase-Transitions in 2 Dimensional Systems. J Phys C Solid State 1973;6(7):1181-1203.
8. Nixon-Abell J, Obara CJ, Weigel AV, Li D, Legant WR, Xu CS, Pasolli HA, Harvey K, Hess HF, Betzig E, Blackstone C, Lippincott-Schwartz J. Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER. Science 2016;354(6311).
9. Wu YM, Whiteus C, Xu CS, Hayworth KJ, Weinberg RJ, Hess HF, De Camilli P. Contacts between the endoplasmic reticulum and other membranes in neurons. P Natl Acad Sci USA 2017;114(24):E4859-E4867.
10. Kozlov MM, McMahon HT, Chernomordik LV. Protein-driven membrane stresses in fusion and fission. Trends Biochem Sci 2010;35(12):699-706.
11. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science 2012;337(6098):1062-1065.
12. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 2013;65(1):36-48.
13. Harrison SC. Viral membrane fusion. Virology 2015;479-480:498-507.
14. Ohashi Y, Munro S. Membrane delivery to the yeast autophagosome from the Golgi-endosomal system. Mol Biol Cell 2010;21(22):3998-4008.
15. Knorr RL, Dimova R, Lipowsky R. Curvature of Double-Membrane Organelles Generated by Changes in Membrane Size and Composition. Plos One 2012;7(3): e32753.

16. Knorr RL, Lipowsky R, Dimova R. Autophagosome closure requires membrane scission. Autophagy 2015;11(11):2134-2137.
17. Tsuboyama K, Koyama-Honda I, Sakamaki Y, Koike M, Morishita H, Mizushima N. The ATG conjugation systems are important for degradation of the inner autophagosomal membrane. Science 2016;354(6315):1036-1041.