Ludger Johannes
Directeur d'unité, DRE INSERM
ludger.johannes@curie.fr
Tel: +33 1 56 24 63 51

Amongst the major classes of biological macromolecules, carbohydrates remain the least well understood when it comes to molecular mechanisms of function. In the Endocytic Trafficking and Intracellular Delivery team, we have formulated the hypothesis that glycan-binding proteins (lectins) from pathogens (Shiga and cholera toxins, polyoma viruses such as SV40, norovirus) or cells (galectins) acquire curvature-active properties (i.e. the capacity to induce and/or sense membrane curvature) in interaction with glycosylated lipids (glycosphingolipids (GSLs), possibly also glycosylphosphatidylinositol (GPI)-anchored proteins) such as to favor their own endocytosis (for pathogenic lectins) or that of cellular proteins (for galectins) via tubular endocytic pits from which so-called clathrin-independent carriers are formed (see ***Figure 1*** for galectins). We term this the GlycoLipid-Lectin (GL-Lect) hypothesis for clathrin-independent endocytic pit construction.

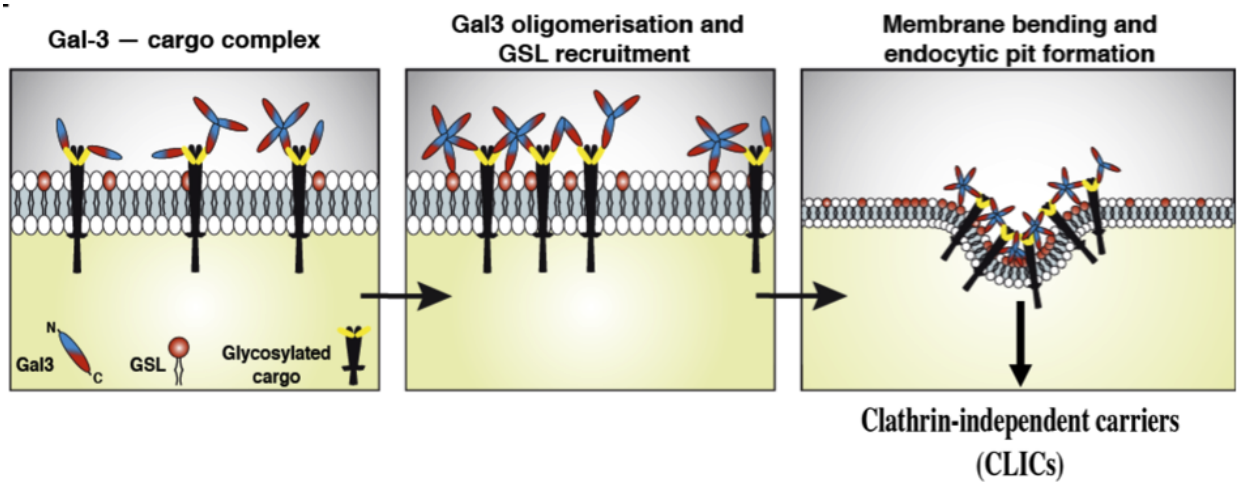

Figure 1*: GL-Lect hypothesis for galectin-3 (Gal3)-driven construction of endocytic pits in the biogenesis of clathrin-independent carriers (CLICs). Monomeric Gal3 is recruited to membranes by binding to glycosylated cargo proteins, such as CD44 and a5β1-integrin. Membrane-bound Gal3 oligomerizes and gains functional glycosphingolipid (GSL) binding capacity, endowing Gal3/GSL complexes with curvature active properties, i.e. the capacity to induce and/or sense membrane curvature. Glycosylated cargoes and lipids are then co-clustered into tubular endocytic pits from which clathrin-independent carriers (CLICs) are formed for endocytic uptake*

into cells. From Lakshminarayan et al., 2014, Nature Cell Biology 16: 595-606.

The GL-Lect mechanism may operate with various glycosylated cargo proteins, which might explain how a small family of galectins (12 members in human) can have very widespread physiological and pathological effects (see *Johannes et al., 2018, J Cell Sci 131: jcs208884* for a review). We are now analyzing how cortical actin dynamics contributes to the clustering of GSL-lectin complexes on active membranes, thereby facilitating the nucleation of endocytic tubules by exploiting membrane fluctuation force and condensation mechanisms that had not been linked before to endocytosis. Furthermore, we are identifying ways by which the GL-Lect mechanism is acutely controlled by growth factor signaling. Finally, we study how GL-Lect domain construction at the plasma membrane programs the intracellular distribution of cargo molecules, notably via the retrograde transport route, thereby exploiting the polarized secretion capacity of the Golgi apparatus for the distribution of cargo proteins to specialized plasma membrane domains in migrating cells (leading edge), epithelial cells (apico-basal sorting and transcytosis), and lymphocytes (immunological synapse). These studies are performed using a combination of cell biological (lattice light sheet microscopy), biochemical (membrane protein purification and reconstitution, glycosphingolipidomics), chemical biology (glycosphingolipid synthesis, small molecule screening), and structural biology (cryo-EM) approaches in model membrane systems, cells, and living organisms.

We also have set out to exploit the specificity of carbohydrate recognition and the ensuing membrane mechanical potential resulting from oligomeric lectin-driven clustering of glycolipids for the development of innovative therapeutic delivery strategies for the treatment of cancer. In collaboration with Prof Eric Tartour (U970 INSERM), we have notably identified the non-toxic poorly immunogenic B-subunit of Shiga toxin (STxB) as a delivery tool to funnel antigenic peptides from tumors of pathogens into the MHC class I and II-restricted presentation pathways of dendritic cells (***Figure 2***).

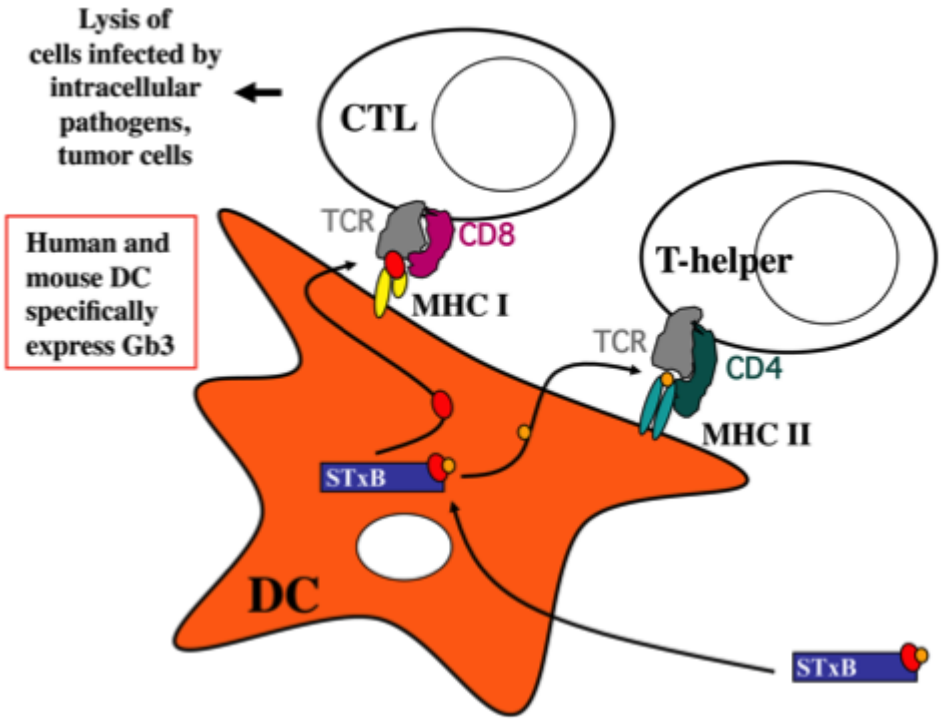

Figure 2: Shiga toxin B-subunit (STxB; represented as blue bar) delivers antigenic peptides (represented as red and yellow circles on STxB) into the MHC class I and II-restricted presentation pathways of dendritic cells (DCs). Exogenously added STxB indeed has been shown to enter the cytosol (for MHC I presentation) and late endosomes/lysosomes (for MHC II presentation) of these cells. The stimulation of a cytotoxic $CD8^+$ T lymphocyte response allows the elimination of tumoral or pathogen-infected cells.

STxB binds to the GSL Gb3, which is expressed by dendritic cells (DCs) of different origins, including human. When associated with tumor antigens, STxB has been shown to induce therapeutic anti-tumor responses in various mouse models, including mucosal head-and-neck carcinomas. We are now using chemical methods to optimize the STxB scaffold for various biomedical applications in immunotherapy, and beyond. 6 patent families have been filed on the STxB technology, 5 of which that have already been delivered. A start-up company creation project is currently ongoing to bring the STxB technology into the clinics.

Another line of research aims at discovering small molecule leads for the development of intervention strategies against protein toxins such as Shiga toxin and ricin, against which no specific treatment exists to date. In collaboration with Daniel Gillet from the French Nuclear Energy Commission CEA, we are developing 2 hit compounds that protect cells and animals against these toxins, and for which the intracellular targets could already be identified.

Biosketch

Ludger Johannes (PhD) is Research Director (DRE) at INSERM. Since the beginning of his biochemistry undergraduate studies in 1987, he is member of the *Studienstiftung des Deutschen Volkes* (German organization of the academically gifted), since 1993 of Boehringer Ingelheim Fonds, since 2012 of the European Molecular Biology Organization (EMBO), and since 2019 of the German Academy of Science — Leopoldina. Between 2001 and 2013, he directed the *Traffic, Signaling and Delivery Team* in the Cell Biology Department (UMR144 CNRS) of *Institut Curie*. Since January 2014, he is heading the *Cellular and Chemical Biology* unit (U1143 INSERM — UMR3666 CNRS). His research aims at establishing fundamental concepts of endocytosis and intracellular trafficking. The Johannes team has made two major contributions in this context: the discovery of a membrane trafficking interface between early endosomes and the Golgi apparatus, and the demonstration that dynamic lectin-induced glycosphingolipid reorganization acts as a driving force for endocytic pit construction in clathrin-independent endocytosis. These studies are very well cited and have been published in several highly visible journals, including *Cell*, *Nature*, *Nature Cell Biology*, and *Nature Nanotechnology*. Between 2014-2020, he was the holder of an ERC advanced grant. He also aims at exploiting his discoveries in fundamental membrane biology research for the development of innovative cancer therapy strategies. His team has validated the B-subunit of Shiga toxin (STxB) as a "pilot" for the delivery of therapeutic compounds to precise intracellular locations of dendritic cells and tumors (12 patent families, 5 of which are delivered in the US, Europe and other countries; creation of biotech companies). Ludger Johannes serves on editorial boards of several international journals (including *PLoS One* and *Traffic*). His team is member of excellence initiative *Cell(n)Scale*.

Key publications

Forrester A, Rathjen SJ, Garcia Castillo MD, Bachert C, Couhert A, Tepshi L, Pichard S, Martinez J, Renard H-F, Valades Cruz CA, Dingli F, Loew D, Lamaze C, Cintrat JC, Linstedt AD, Gillet D, Barbier J, Johannes L (2020) Functional dissection of the retrograde Shiga toxin trafficking inhibitor Retro-2. Nat Chem Biol 16: 327–336

Comments: Hesso Farhan, Nature Chemical Biology volume 16, pages 229–230 (2020)

Watkins EB, Majewski J, Chi EY, Gao H, Florent JC, Johannes L (2019) Shiga toxin induces lipid compression: a mechanism for generating membrane curvature. **Nano Lett** 19: 7365-7369

Johannes L, Pezeshkian W, Ipsen JH, Shillcock J (2018) Clustering on membranes – Fluctuations and more. Trends Cell Biol 28: 405-415

Pezeshkian& W, Gao&# H, Arumugam&# S, Becken# U, Bassereau P, Florent JC, Ipsen* JH, Johannes* L, Shillcock* J (2017) Mechanism of Shiga toxin clustering on membranes. ACS Nano 11: 314-324 (& co-first authors, # authors from Johannes group, * principal investigators and corresponding authors)

Comments:
http://www.healthcanal.com/infections/76071-forces-at-play-a-new-infection-route-for-bacteria.html

Shafaq-Zadah M, Gomes-Santos CS, Bardin S, Maiuri P, Maurin M, Iranzo J, Gautreau A, Lamaze C, Caswell P, Goud B, Johannes L (2016) Persistent cell migration and adhesion rely on retrograde transport of beta1 integrin. **Nat Cell Biol** 18: 54-64

Comments: F1000 Prime Recommended

Bhatia# D, Arumugam# S, Nasilowski M, Joshi H, Wunder# C, Chambon# V, (…), Johannes* L, Dubertret* B, Krishnan* Y (2016) Quantum dot-loaded monofunctionalized DNA Icosahedra for single particle tracking of endocytic pathways. Nat Nanotechnol 11: 1112-1119 (* corresponding authors and principal investigators; # authors from Johannes team)

Comments: Nature Biotechology, 2016, Vol. 34, number 10, page 1036

http://www.hfsp.org/frontier-science/awardees-articles/magic-bullet-dna-wrap-long-duration-imaging

Renard H-F, Simunovic M, Lemière J, Boucrot E, Garcia-Castillo MD, Arumugam S, Chambon V, Lamaze C, Wunder C, Kenworthy AK, Schmidt AA, McMahon H, Sykes C, Bassereau P, Johannes L (2015) Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. Nature 517: 493-496

Comments: Nature News and Views: Haucke V, 2015, **Nature** 517: 446-447 ; Nat Rev Mol Cell Biol, published online 15 January 2015; 16(2): 68 ; F1000Prime Recommended

Johannes L, Parton RG, Bassereau P, and Mayor S (2015) Building endocytic pits without clathrin. Nat Rev Mol Cell Biol 16: 311-321 1

Lakshminarayan R, Wunder C, Becken U, Howes MT, Benzing C, Arumugam S, Sales S, Ariotti N, Chambon V, Lamaze C, Loew D, Shevchenko A, Gaus K, Parton RG, Johannes L (2014) Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers. Nat Cell Biol 16: 595-606

Comments: Nat Cell Biol, June 2014, Volume 16 No 6 pp506-507 ; Nat Rev Mol Cell Biol, published online 11 June 2014 ; Science Editor's Choice 2014, VOL 344, ISSUE 6188, PAGE 1129

Römer W, Pontani LL, Sorre B, Rentero C, Berland L, Chambon V, Lamaze C, Bassereau P, Sykes C, Gaus K, Johannes L (2010) Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis. Cell 140: 540-553

Comments: Faculty of 1000 Biology, category must read

Cell Video abstract at http://www.youtube.com/watch?v=SNWMpPckBU0

Stechmann# B, Bai# SK, Gobbo E, Lopez R, Merer G, Pinchard S, Panigai L, Tenza D, Raposo G, Beaumelle B, Sauvaire D, Gillet* D, Johannes* L, Barbier J (2010) Inhibition of retrograde transport protects mice from lethal ricin challenges. Cell 141: 231-242 (* corresponding authors, # authors from Johannes team)

Comments: Seaman and Peden, 2010, Cell 141: 222-224 ; Nature 2010, Vol 464, page 1106 ; Science editor's choice Volume 328, Number 5981, Issue of 21 May 2010

Ewers H, Römer W, (…), Helenius A, Johannes L (2010) GM1 structure determines SV40-induced membrane invagination and infection. Nat Cell Biol 12: 11-18

Comments: Nat Rev Mol Cell Biol 2010 Vol 8, pp 87

Römer W, Berland L, Chambon V, Gaus K, (…), Lamaze C, Raposo G, Steinem C, Sens P, Bassereau P, Johannes L (2007) Shiga toxin induces tubular membrane invaginations for its uptake into cells. Nature 450: 670-675

Comments: Nat Rev Mol Cell Biol 2008 Vol 9, pp 2 ; Nat Rev Microbiol 2008 Vol 6, pp 92 ; Faculty of 1000 Biology, category must read

Mallard F, Tang BL, Galli T, Tenza D, Saint-Pol A, Yue X, Antony C, Hong WJ, Goud B, Johannes L (2002) Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform. J Cell Biol 156: 653-664

Key publications

Year of publication 2020

Forrester A, Rathjen SJ, Garcia Castillo MD, Bachert C, Couhert A, Tepshi L, Pichard S, Martinez J, Renard H-F, Valades Cruz CA, Dingli F, Loew D, Lamaze C, Cintrat JC, Linstedt AD, Gillet D, Barbier J, Johannes L (2020 May 29)
Functional dissection of the retrograde Shiga toxin trafficking inhibitor Retro-2
Nature Chemical BiologyNature : 16 : 327,336 : DOI : 10.1038/s41589-020-0474-4

Year of publication 2019

Erik B. Watkins, Jaroslaw Majewski, Eva Y. Chi, Haifei Gao, Jean-Claude Florent, Ludger Johannes (2019 Sep 20)
Shiga Toxin Induces Lipid Compression: A Mechanism for Generating Membrane

Curvature
Nano letters : 19 : DOI : 10.1021/acs.nanolett.9b03001

François Legoux, Déborah Bellet, Celine Daviaud, Yara El Morr, Aurelie Darbois, Kristina Niort, Emanuele Procopio, Marion Salou, Jules Gilet, Bernhard Ryffel, Aurélie Balvay, Anne Foussier, Manal Sarkis, Ahmed El Marjou, Frederic Schmidt, Sylvie Rabot, Olivier Lantz (2019 Aug 31)
Microbial metabolites control the thymic development of mucosal-associated invariant T cells.
Science (New York, N.Y.) : DOI : eaaw2719

Year of publication 2018

Johannes Ludger, Pezeshkian W, Ipsen JH, Shillcock J (2018 May 7)
Clustering on Membranes: Fluctuations and More
Trends Cell BiolTrends Cell Biol : 28 : 405 415 : DOI : 10.1016/j.tcb.2018.01.009

Year of publication 2016

Daniela Chmiest, Nanaocha Sharma, Natacha Zanin, Christine Viaris de Lesegno, Massiullah Shafaq-Zadah, Vonick Sibut, Florent Dingli, Philippe Hupé, Stephan Wilmes, Jacob Piehler, Damarys Loew, Ludger Johannes, Gideon Schreiber, Christophe Lamaze (2016 Dec 6)
Spatiotemporal control of interferon-induced JAK/STAT signalling and gene transcription by the retromer complex.
Nature communications : 13476 : DOI : 10.1038/ncomms13476

Mijo Simunovic, Emma Evergren, Ivan Golushko, Coline Prévost, Henri-François Renard, Ludger Johannes, Harvey T McMahon, Vladimir Lorman, Gregory A Voth, Patricia Bassereau (2016 Oct 4)
How curvature-generating proteins build scaffolds on membrane nanotubes.
Proceedings of the National Academy of Sciences of the United States of America : 113 : DOI : 10.1073/pnas.1606943113