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 $\alpha5\beta1$-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.
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


Comments: Faculty of 1000 Biology, category must read
Cell Video abstract at http://www.youtube.com/watch?v=SNWMpPckBU0


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


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


Key publications

Year of publication 2020


Year of publication 2019

Curvature

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


Microbial metabolites control the thymic development of mucosal-associated invariant T cells.


Year of publication 2018

Johannes Ludger, Pezeshkian W, Ipsen JH, Shillcock J (2018 May 7)

Clustering on Membranes: Fluctuations and More


Year of publication 2016


Spatiotemporal control of interferon-induced JAK/STAT signalling and gene transcription by the retromer complex.

Nature communications : 13476 : [DOI: 10.1038/ncomms13476]


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]