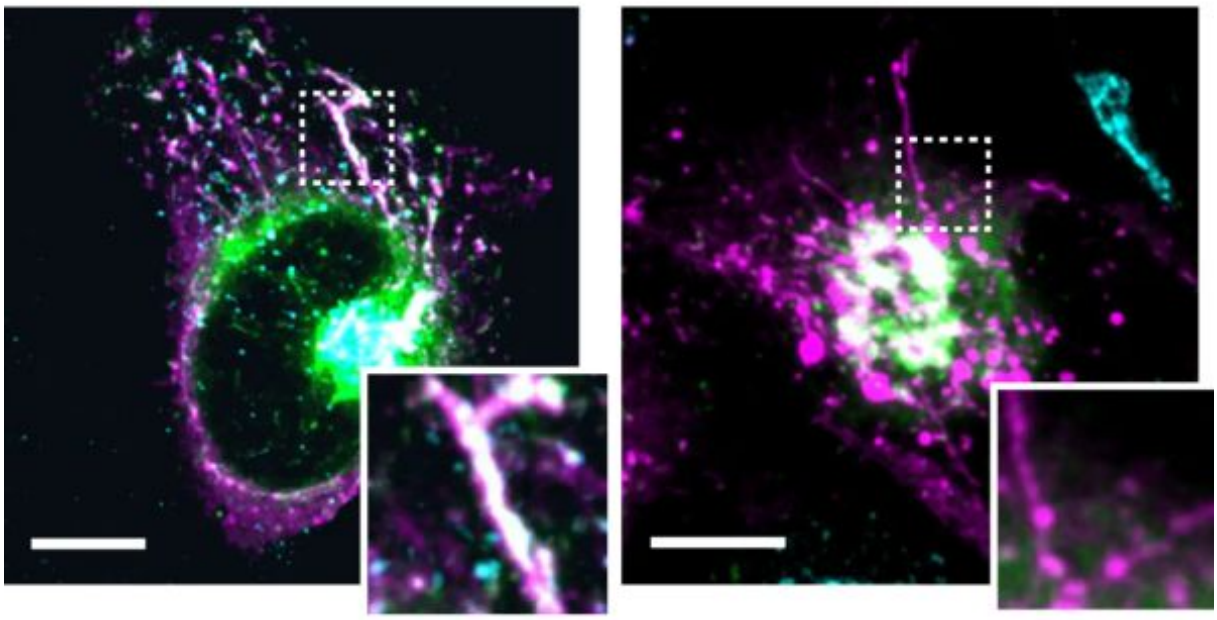


Shedding light into protein sorting in cells

In a new study published in eLife, researchers provide a first experimental demonstration of the role of the protein TGN46 in the sorting process of secretory proteins, identifying the region that encodes for this function using quantitative fluorescence microscopy and mutagenesis.

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Secretory proteins control various essential processes such as immunity, metabolism and cellular communication, playing a key role in many diseases such as cancer or neurological disorders. They are synthesized in the endoplasmic reticulum organelle and travel to the trans-Golgi network (TGN). This cellular compartment works like a sorting station that regulates the flow within the cell, organizing newly synthesized proteins and other molecules in different transport vehicles and directing them to their final destinations. As in a factory assembly line, each cargo molecule is processed and packaged into vesicles, that are timely and orderly guided to the correct cellular compartment or exported out of the cell, preventing congestion and ensuring proper cellular function.

The targeting signals and molecules that participate in this sorting process, driving each protein to the correct destinations are still unclear and a subject of discussion. Previous studies have pointed out that one of the key players might be a transmembrane protein, known as the protein TGN46, that cycles quickly between the trans-Golgi network and the plasma membrane, transported to the cell surface by vesicles that also typically carry secretory proteins. However, its specific role in this sorting process had not been shown yet.

Investigating the TGN46 role experimentally

Now, ICFO researchers **Pablo Lujan, Felix Campelo**, **Javier Vera** and **Prof. Maria Garcia-Parajo**, together with researchers from the Institute for Research in Biomedicine and the Center for Genomic Regulation in Barcelona, the Tokyo University of Pharmacy and Life Sciences and the Pompeu Fabra University, published a study in eLife showing that the protein TGN46 plays a key role in sorting the cargo proteins into their transport carriers at the TGN and that this role is described by the part of the molecule located inside the trans-Golgi network.?

To investigate the function of the TGN46 protein, the team studied two types of cells, wit and without the protein, and measured the amount of secretion of a specific secretor protein - named PAUF. Using immunofluorescence microscopy, they saw that the mutan cells without TGN46 secreted 75% less protein. Moreover, they used confocal fluorescenc microscopy to assess how many containing vesicles were in each type of cell, seeing that th mutants had much fewer vesicles, and measured the export rate of the secretory protein wit a microscopy technique named FLIP, the acronym for fluorescence loss in photobleachin microscopy

They also observed that this secretory PAUF protein was present in the membrane tubules o the normal cells, but not in the mutant ones. All of these findings indicated that the cell without TGN46 couldn't complete the sorting and loading of the secretory proteins into thei transport vesicles.

? Researchers also intended to find out which parts of the TGN46 receptor were in charge f the sorting and packaging functions and found that only the luminal domain, that is the pa t of the receptor facing the inside of the Golgi, was necessary to complete the proces

Exploring the sorting mechanism further

The article provides the first experimental confirmation that the protein TGN46 works as a cargo receptor, sorting secretory proteins in the trans-Golgi network, which are then packaged into carrying vesicles, transported to the surface or secreted outside the cell. Researchers suggest that future efforts could be placed into investigating which other secretory proteins are handled by TGN46. i¿½The next steps we want to pursue are, first, to find the list of proteins that are secreted following this route, which could, in the long term, open new therapeutic options for diseases related to abnormalities in their secretioni¿? , points ou**Felix Campelo**, ICFO researcher and one of the authors of the study. i¿½Second,

from a more biophysical perspective, we want to understand the mechanism by which TGN46 sorts and loads these proteins into the transport carriers, because preliminary evidence suggests that the ability of TGN46 to form biomolecular condensates may play a role in its function."