

Post-ER trafficking in Bergen, Norway

Discrete Structures

Cargo of the biosynthetic secretory pathway doesn't always follow classical transport routes via the Golgi apparatus. There had to be a central station that sends the proteins onto their right paths and Jaakko Saraste found it.

Just when you thought you knew everything about the structure of a cell, along comes a completely new organelle. In school, everyone learns that a eukaryotic cell consists of the cell membrane, the nucleus, some cytoplasm, the endoplasmic reticulum (ER), the Golgi apparatus and so on and so forth. You may also have learned that two of these organelles, the ER and the Golgi apparatus, are the major players in the biosynthetic-secretory pathway. In this pathway, proteins are translated by ribosomes within the rough ER and then transported to the Golgi where they are processed and matured before they reach their final destination at the

Center (MIC), Swiss Master student Sarah Bazzocco, and former PhD student Ragna Sannerud who, "in spite of her completely Norwegian name, is completely French", focus their main research interest on pre-Golgi trafficking.

Immediate control

After newly-synthesized proteins and lipids leave the ER at specialised exit sites, they enter the so-called intermediate compartment (IC), which, according to Saraste, is a permanent, multifunctional membrane network. This network consists of two very different domains, a vacuolar domain, "the pre-Golgi vacuole (*Cell* 38 (2),

535-49), which develops special luminal conditions, such as low pH, and is the sorting unit" and a tubular domain, which is a "dynamic network that connects the vacuoles and functions in Golgi biogenesis but also has other roles", Saraste explains. The tubules, furthermore, connect the intermediate compartment with the cell periphery and, in this way, could allow for Golgi-independent trans-

port mechanisms, for example, the biosynthetic trafficking of cholesterol to the cell surface (*Mol. Biol. Cell* 17 (4), 1514-26).

But what makes this small space between ER and Golgi a whole new organelle and not just a stopover between points A and B? Saraste elucidates, "The autonomous nature of the IC becomes apparent when the Golgi is 'knocked down' by the fungal antibiotic brefeldin A (BFA). Under these conditions the tubular IC network remains coupled to the centrosome, the main

microtubule organising centre of the cell, and continues to act as a connector between the central and peripheral parts of the cell" (*Mol. Biol. Cell* 20 (2), in press).

The two IC domains can be easily identified immunocytochemically by the marker Rab1, a GTPase from the Ras superfamily, which regulates the transport of post-ER carriers. Rab1 has two isoforms, Rab1B can be found in the vacuolar domain, whereas Rab1A has a higher affinity to the tubular domain of the IC. It was exactly this isoform, which led to the discovery of a novel IC subdomain, the pericentrosomal intermediate compartment, short pIC.

Hide and seek

With the help of stably transfected, normal rat kidney cells and time-lapse confocal microscopy, Saraste and his group were able to show large Rab1A-positive membrane clusters at the cell centre, located next to the centrosome. This novel structure, which is usually masked by the Golgi ribbon, becomes clearly visible when the centrosome is relocated, e.g. during cell division or migration.

"When we watched the Rab1A-containing pIC emerge from behind the Golgi in living cells, the first reaction was pure astonishment," Saraste recalls. This astonishment was even greater when it became clear that the pIC participates in the transport of secretory cargo, as evidenced by the temperature-sensitive mutant of Semliki Forest virus, SFV ts-1. Interestingly, proteins of the transport machinery including the coat protein COPI, the transport receptor KDELR and the Rab1 effectors p115 and GM130 can also be found in the newly discovered structure, but this is not yet the end of the line for Saraste. "We need to know more about the molecular composition of these membranes, for example, to facilitate their ultrastructural analysis."

Another compartment, which, in many cell types, is similarly localised near the centrosome and close to the Golgi stacks and the trans-Golgi network, is the endocytic recycling compartment, ERC. Could there be



From the left: Michaël Marie, Hege Dale, Jaakko Saraste, Sarah Bazzocco

plasma membrane. But has someone ever wondered what's happening between the sheets, or in this case between the membrane network of the ER and the cisternal stacks of the Golgi apparatus? At least one person has, Professor Jaakko Saraste from the Department of Biomedicine at the University of Bergen, Norway.

He and his "truly pan-European group" consisting of himself, a Finn, French post-doc Michaël Marie, Hege Dale, a Norwegian Chief Engineer at the Molecular Imaging

a functional link between these two neighbouring compartments? Saraste points out that “a direct IC-endosome connection is of interest regarding the operation of secretory pathways in different cell types, and provides a new angle to think about Golgi biogenesis and signalling between endomembranes and the nucleus in mammalian cells. It is interesting to note that both structurally and in terms of the molecular machinery, this pathway looks very similar to the secretory pathway of the yeast *Saccharomyces cerevisiae*” (*Cell Mol. Life Sci.* 65, 2859-74). And indeed, when Saraste and his team studied the endocytic recycling of transferrin in cells treated with BFA, which also affects certain clathrin-mediated transport steps, they found that instead of its endosomal pathway, transferrin can use the BFA-resistant pathway via the pcIC and the tubular system of the IC to travel back to the plasma membrane, indicating that the ERC and pcIC are functionally coupled and form a “pericentrosomal membrane system”.

the traffic charts of mammalian cells would be in place,” says Saraste. “I’m sure that an increased appreciation of Golgi-independent transport routes will, in due course, add new arrows to these charts.”

To sum it all up, the novel subdomain of the intermediate compartment, the pcIC, can be understood, according to Saraste, as a “central station, directing molecules either to the Golgi or to pathways that bypass this organelle”.

Norwegian hospitality

But aside from all the groundbreaking research, however does a Finn end up in Norway, of all countries, where everything’s a bit more expensive and somewhat stricter than in Finland? Saraste tells his story. “In the spring of 1991, I participated in a cytoskeleton meeting that took place in a hotel by the Hardanger fjord. On the second-to-last day, after the last scientific session, I was on my way to the farewell party but wasn’t quite sure where this happen-



Secretly hiding behind the Golgi ribbon the pericentrosomal intermediate compartment only comes out of the dark when the centrosome is relocated

However, not only the endosomal system can do perfectly well without the Golgi apparatus but there are also certain proteins that, after their synthesis in the ER, bypass this organelle on their way to the plasma membrane. One of them is the cystic fibrosis transmembrane conductance regulator, CFTR. Mutations in the CFTR gene are the cause for cystic fibrosis and congenital absence of the vas deferens. Until now, it was speculated that this chloride channel uses some unconventional endosomal pathway but the Saraste group was able to show that the pcIC plays a major role in the Golgi-independent trafficking of CFTR (*Mol. Biol. Cell* 20 (2), *in press*).

Stations and pathways

These newly discovered compartmental organisations of the secretory pathway, connections between existing membrane systems and transport mechanisms, especially routes bypassing the Golgi apparatus, become a more and more fascinating area of research and will also have an impact on future cell biology text books. “It was clear from the start that some major revisions in

ing was going to take place. So, I faithfully followed some people down the stairs and ended up in a small hotel room in the middle of a cheerful group of biochemists from Bergen, who were having a “vorspiel”, a kind of warm-up party with own drinks and snacks. I later learned that this type of “pre-party” is a tradition in Norway due to the high price of alcoholic beverages. Despite the fact that I was a total stranger they asked me to join in and, without hesitation, I sat down on the floor next to the person closest to me. So, thanks to the strict alcohol policy of the Norwegian government I met Anni Vedeler. A year later I moved from Stockholm to Bergen, where I first got a temporary appointment as a visiting scientist with the help of the biochemistry professor Torgeir Flatmark. Shortly after that, Anni and I got married.”

And so it seems that Norway, the land of the midnight sun, is not only a good place to find life-long friends and partners but maybe an even better place to do outstanding research, which will provide new insight into the fascinating world of post-ER trafficking.

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