Electrified regulated protein transport

Physiologists have for a long time regarded the communication between cells as a purely "external" process. However, research carried out by Dr. Nikolaj Klöcker and his team at the Freiburg University Medical Centre now shows that the cells do not exclusively regulate their electrical properties directly at the cell membrane. They also found a range of molecular switches in neurons and epithelial cells that are able to control the cells' electrical properties. These switches not only influence the synthesis, but also the intracellular transport and degradation of receptors and ion channels that enable the exchange of information on the cell surface.

Nerve and muscle cells need to communicate with each other. This communication is achieved through electrical current, i.e. electrical charges in the form of sodium and potassium ions. The permeability of the cell membrane for these ions determines the excitation state of cell, i.e. the sensitivity with which it reacts to the message from a neighbouring cell and whether it can respond in an adequate form. The strength of the ion flow is mediated by ion channels – large protein complexes that form transmembrane pores and allow the carriers of electrical charge to pass through. "Electrophysiologists have long concentrated on the investigation of the opening and closing of individual ion channels and their regulation through modulators on the cell membrane," said Dr. Nikolaj Klöcker of the Institute of Physiology (Department II) at the Freiburg University Medical Centre. "However, the fact that the number and distribution of ion channels and membrane receptors have an effect on the ion flow also needs to be taken into account."

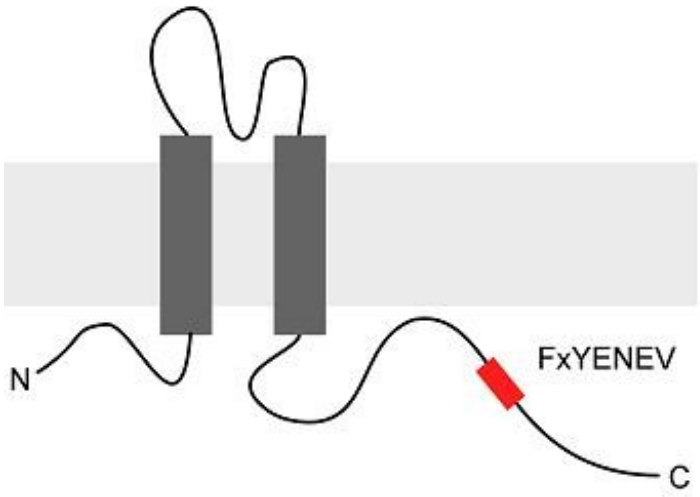
From station to station

Klöcker and his team are focusing on physiological experiments and cell biology. They are trying to find out what processes cells use to regulate the number of channel proteins and receptors on their surfaces. This is an important issue because defective regulation can lead to diseases such as cystic fibrosis and diabetes mellitus. There are three theoretical possibilities: First, cells can regulate the synthesis rate of molecules. Enhanced production increases the availability of certain molecules and hence their number on the cell membrane. Second, it could be envisaged that the degradation of membrane proteins on the cell surface occurs. There is evidence that molecules are constantly removed from the membrane and recycled inside the cell. And the third option is that cells control the transport of ion channels and receptors to the surface. Over the last few years, Klöcker and his team have particularly focused on the latter two possibilities.

The precursors of protein complexes that are determined for the cell membrane have to pass a

range of stations in a cell where they are then assembled into the final product. The transport of membrane proteins involves several intermediary steps. The first station is the endoplasmic reticulum (ER), where amino acids are synthesised and where they fold into a three-dimensional protein. Vesicles then transport the proteins to the Golgi apparatus where sugar residues, which specify the later function of the protein, are attached. The final proteins then leave the Golgi apparatus and are transported to the cell membrane, once again packaged in vesicles. When the proteins are no longer being used, for example when the cell wants to decrease its electrical conductivity, then a third type of vesicle will transport them back into the cell where they are degraded by proteasomes. "We have discovered that all three stations involve specific control and regulation mechanisms that enable the cells to influence the number of proteins located on the cell surface," said Klöcker.

A passport for the outward journey



The schematic shows the Kir2.1 membrane channel. A sequence motif (-FxYENEV-, shown in red), flanked by hydrophobic amino acids, is essential for the selective export of Kir2.1 from the endoplasmic reticulum (ER). N and C denote the N and C terminus of the protein.
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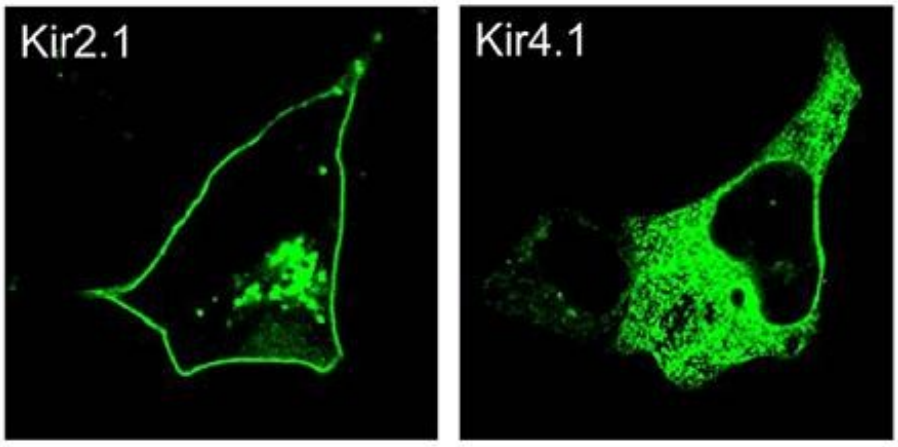
An example of such a regulation is the export of the Kir2.1 potassium channel from the ER towards the Golgi apparatus. Kir2.1 increases the membrane conductivity for potassium ions and contributes to the creation of the resting membrane potential of muscle cells and neurons. "Working in cooperation with another group of researchers we have for the first time ever been able to show that membrane proteins in mammalian cells, i.e. the Kir2.1 ion channel, have a specific sequence motif that increases their probability of being transported to the Golgi apparatus," said Klöcker. This protein segment, which can be compared to a passport, binds to an envelope protein complex that recognises the labelled amino acid sequence and directs it to the ER exit sites, where the envelope complex encloses the Kir2.1 molecules and interacts with the ER membrane, leading to the formation of a transport vesicle that subsequently transports the freight to the Golgi apparatus. Without such export signals, a membrane protein would only be able to enter the vesicles by chance and the transport rate of proteins would be much lower.

Klöcker and his team of researchers have discovered another export sequence in the Kir2.1 channel protein that increases the export rate of the protein from the Golgi apparatus to the cell membrane. Besides these two pieces of evidence that point to a regulated anterograde transport, Klöcker and his team have also been able to verify that the recycling of certain membrane proteins



Subcellular distribution of the potassium channels Kir2.1 and Kir4.1 in opossum renal epithelial cells, each coupled to green fluorescent protein (GFP). Kir2.1 has a specific export sequence, which enables it to be efficiently transported out of the endoplasmic reticulum (ER), subsequently accumulating in the Golgi apparatus, which is located close to the cell nucleus, and at the plasma membrane. Kir4.1 does not have such a sequence and therefore remains predominantly in the ER (right).
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is regulated. They found that the expression of the channels that are responsible for the pacemaker function of specialised heart muscle cells can be regulated on the cell surface through external signals, such as the hormone angiotensin II. The hormone initiates a complicated signalling cascade inside the cells, which in turn causes channels that were previously removed from the membrane and temporarily stored in membrane reservoirs, to migrate back to the membrane. "It is interesting to note that patients who suffer from chronic cardiac insufficiency have high local angiotensin II concentrations and an increased current through these pacemaker channels, thereby leading to cardiac arrhythmia," says Klöcker, who additionally assumes that lower angiotensin II concentrations have the opposite effect.

More detailed investigations to follow

In order to advance their research, Klöcker and his team now need to look for the molecular interaction partners of the transport sequences they have discovered. This will help them clarify the molecular mechanisms underlying the transport of proteins as well as identify the molecules that mediate the fine control. The first step into this direction has already been made in the form of a study that was published earlier this year in the renowned scientific journal Science. Teams led by Klöcker and Prof. Dr. Bernd Fakler, the director of the Department II at the Institute of Physiology, used proteome analyses to discover two new interaction partners of an important glutamate receptor in brain neurons. This glutamate receptor mediates the rapid excitatory communication between neurons and contributes, amongst other things, to the brain's ability to react plastically to environmental stimuli. In addition, the newly identified interaction partners modulate, amongst other things, the number of glutamate receptors on the cell surface. However, it is still not known which partial step in the transport of receptors is affected by these interaction partners. "Therefore, we will have to use proteome analyses to have a closer look at the membranes of the different transport stations in cells," said Klöcker. The researchers plan to isolate ER, Golgi and plasma membranes from neurons, purify the membrane protein complexes and identify attached interaction partners using mass spectrometry. "The subcellular fine resolution is currently our next big goal," said Klöcker.

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