English summary for the non-expert

The cell nucleus, in which the chromosomes are stored, is surrounded by the nuclear membrane. For the communication and transport between the nucleus and the remainder of the cell, pores are thus necessary. In 1950, Callan and Tomlin suggested the existence of large pores in the nuclear membrane. They saw these pores using an electron microscope. Now 65 years later we know a lot more of these pores, which we call Nuclear Pore Complexes or NPCs. With a multitude of new techniques, scientists have studied what the NPCs consists of, how they are assembled and how they work. In short we can say that NPCs consist of about 500 proteins. About two-thirds of them together form a ring-shaped structure embedded in the nuclear membrane. Inside the ring are filament-like proteins. Rapid transport through the NPC requires energy and is provided by chaperone proteins that can bind the filaments. Many of the smaller molecules can pass without the chaperone proteins, but this happens at a slower rate. What determines whether a molecule goes to the nucleus? The Nobel Prize in physiology was given in 1999 to Günter Blobel. He described, among other things, that the proteins have a special address code that targets them to the nucleus, a "nuclear localization signal" or NLS. This NLS is recognized by the chaperone proteins that facilitate the rapid transport.

The above knowledge focused on the transport of soluble proteins. These are proteins that function in the aqueous environment of the cell. However, there are also proteins which function in the membranes, so-called membrane proteins. In the context of the nucleus, this concerns the membrane proteins that function in the nuclear membrane. The nuclear membrane consists of two membranes, the inner membrane and the outer membrane. The starting point of my research was that we asked the question: are there also for membrane proteins such NLS codes and do they help to go through the NPC into the inner membrane?

I studied two proteins from baker's yeast: called Heh1 and Heh2. These two proteins are made at the outer membrane, but function in the inner membrane of the nucleus. They should therefore go through the NPC in one way or the other. Researchers from the US, Megan King, Patrick Lusk and the aforementioned Günter Blobel showed...
that Heh1 and Heh2 carry an NLS and use of chaperones to get through the NPC. In chapter two I and my colleagues showed that an NLS was not enough. In addition to the NLS is also a long linker needed. The linker and the NLS together are thus the address code for these two membrane proteins. The linker part in the address code is very thin, long and flexible and ensures that the NLS may be located at a distance from the membrane. We proposed that the linker is of importance for the binding of the chaperones and the filaments, which are located at some distance from the membrane.

Not only the linker part of the address code is special, also the NLS of Heh1 and Heh2 have special features. What is so special about this NLS compared to previously described NLSs I examined in chapter 3. The main difference is that, especially the NLS of Heh2, is able to bind the chaperone Kap60 in the absence of the chaperone Kap95. For many other NLSs, Kap60 can only properly be bound if also Kap95 is in the complex. One specific position in the NLS is responsible for this special way of binding. It could be that this adaptation of the NLS also has to do with the fact that membrane proteins have more difficulty binding the chaperones as compared to soluble proteins. Thus, to be able to compete with the soluble proteins they may have super-NLS (and linkers). Other explanations are not excluded.

How Heh1 and Heh2 go through the NPC is difficult to prove. The road through the NPC for the membrane proteins is probably not easy because the linker with NLS and chaperones must move through narrow openings. In order to further map how the proteins pass through the NPC, in chapter 4 we made use of yeast cells that lacked portions of the ring-shaped structure of the NPC. I saw that the membrane proteins still made it to the inner membrane through these mutated NPCs, but less well. It was striking that the mutations of the NPC had a greater effect on the transport of the membrane proteins than on the transport of the soluble proteins. Apparently an intact ring structure is of greater importance for the transport of the membrane proteins.

It is currently unclear why the mechanism of regulation of the amount of Heh1 and Heh2 in the inner membrane exists at all. In Chapter 5, I worked on the question of why the cells are sick and make a deformed nucleus membrane as a cell makes too much of the membrane proteins that enter the inner membrane. We tested a number of possibilities. One was the possibility that the aforementioned competition for
chaperones perhaps was the problem. This was not the case and the question is still unresolved.

In the discussion section I will explain how my findings compare to those of others and I discuss alternative interpretations of my work.

All in my entire thesis contributes to our understanding of how a membrane protein can find its way to the nucleus. The work was done in yeast, but it is expected that such a mechanism may also be of interest in human cells. For example, we already know that the exact composition of the inner membrane is different in cells of different tissues. In general it can be said that history shows that basic knowledge about the biology, as described in this thesis, can be of great value to humanity in unpredictable manners and timeframes.