6 Summary

In multicellular organisms, epithelia and endothelia form barriers to maintain the function of organs, but also to prevent the passage of drugs. To prevent diffusion of water soluble molecules through the paracellular pathway, the intercellular space between adjacent epithelial or endothelial cells is sealed by tight junctions (TJ). TJs appear in freeze-fracture replicas as intramembranous networks of strands on the apical end of the lateral surface of the cells. Structural backbone of the TJs in the specifically tight blood-brain barrier are the claudins (cld) 3 and 5. Cld5 is sealing the TJs of the blood-brain barrier for molecules <800 Da. In this thesis it is hypothesized that the TJ strands are formed and the paracellular space is tightened by homo- and heterophilic *cis* and *trans* interactions of claudins. The molecular interaction mechanism between claudins has been unknown.

The results demonstrate direct homophilic self-association of the second extracellular loop (2.ECL) of claudins 3 and 5 in size exclusion chromatography. Systematic amino acid substitutions of cld5 showed, that the 2.ECL is involved in homophilic *trans*-interactions, but not in *cis*-interactions. Molecular determinants important for *trans*-interaction were identified. HEK-293 cells were established as TJ-free cell system for the analysis of homophilic *cis*- and *trans*-interaction, as well as strand formation by cld5, independent from the influence of endogenous claudins. The *trans*-interaction ability, analysed with confocal microscopy, and the subcellular localisation of the Cld mutants, determined by cell surface biotinylation and confocal microscopy, leads to structural models of a cld5-monomer and a homophilic cld5-*trans*-dimer. The sequence-homolog part of the structure of a hypothetical bacterial protein (BB2244) was assigned to the 2.ECL of cld5. According to this, the 2.ECL of cld5 (mouse) shows a helix-turn-helix motif, which is stabilized by the turn-conformation formed by a network of hydrogen bonds, the two proline P150 and P153, as well as hydrophobic side chain interactions between V152 and L160. In the homodimer model, the 2.ECLs *trans*-interact antiparallelly through aromatic residues (F147, Y148, Y158). A potential binding partner for the *trans*-interacting amino acids Q156 and E159 remain to be identified. The structural models are supposed to be adapted to claudins with high sequence homology to cld5, for the first time, defined as classical claudins 1-10, 14, 15, 17 and 19.

Moreover, the strand formation was analysed with respect to *cis*- and *trans*-interactions. Freeze-fracture electron microscopy shows a strongly reduced number of intramembranous strands in mutants with disturbed *trans*-interaction although the *cis*-interaction is unimpaired. A mechanism is postulated which explains the morphology of cld-5-based discontinuous TJ strands. According to this, the particles in the discontinuous strands of cld5 *trans*-interact with

the particles of the opposing cell to form continuous TJ strands in the paracellular space. We propose that these particles are composed by cld5-hexamers.

Another aspect of this thesis is the investigation of the interaction between the 2.ECL of claudins and *Clostridium perfringens* enterotoxin (CPE). As shown by the analysis of peptide array libraries, CPE is interacting with peptides from the 2.ECL of claudins 3, 6, 7, 9 and 14, but not with 2.ECL of claudins 1, 2, 4, 5, 6, 8, 10-13, 15, 18, 19, 20 and 22. Substitution analysis, using peptide array libraries, identified the turn motif NPLVP as essential for interactions between CPE and the isolated 2.ECL of claudins. In contrast, the turn motif seems to be of no relevance for the interaction of CPE with native full-length Cld-5 molecules on the cell surface. This suggests that the binding-relevant structure of the 2.ECL from Cld5 depends strongly on the membrane topology. Incubations of transfected HEK-293 cells with CPE reveal two amino acids substitutions in the 2.ECL of Cld5, T151A and Q156E, that are mainly involved in the Cld-CPE-interactions. It is also shown that CPE does not bind directly to the TJs reconstituted by cld3 or cld5 expressed in HEK-293 cells, but binds to free cld3 or cld5 molecules on the plasma membrane. Hence, the CPE-induced opening of TJs is likely due to an imbalance of polymerisation and depolymerisation of the Cld strands.

Overall, these investigations lead to a better understanding of the TJ structure. The characterisation of the Cld-CPE and the Cld-Cld-interactions is thought to contribute to the design of peptides or low molecular weight substances for a direct modulation of the TJs to enhance the drug delivery to the blood-brain barrier.