Possible roles of LI-cadherin in the formation and maintenance of the intestinal epithelial barrier

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LI-cadherin belongs to the so called 7D-cadherins, exceptional members of the cadherin superfamily which are characterized by seven extracellular cadherin repeats and a small cytosolic domain. Under physiological conditions LI-cadherin is expressed in the intestine and colon in human and mouse and in the rat also in hepatocytes. LI-cadherin was shown to act as a functional Ca²⁺-dependent adhesion molecule, linking neighboring cells and a lot of biophysical and biochemical parameters were determined in the last time. It is also known that dysregulated LI-cadherin expression can be found in a variety of diseases. Although there are several hypothesis and theoretical models concerning the function of LI-cadherin, the physiological role of LI-cadherin is still enigmatic.

The Simple Epithelial Barrier and the Role of Cell-Cell-Adhesion

Epithelia cover inner and outer surfaces of the body of eumetazoic animals. Thus, epithelia represent the primary barrier for controlled transport of water or dissolved molecules into or out of the body. For example, the intestinal mucosa contains a highly dynamic simple epithelium that fulfills the selective uptake of nutrients and ions, the resorption of water and the prevention of pathogen invasion. It has to be emphasized that controlled transport in this context means that on one side unwanted loss of water, ions or nutrients must be avoided. On the other side, necessary nutrients have to be absorbed and thus these molecules must be transported over the cellular barrier. For this process, there are two main transport pathways: transcellular and paracellular. In the first case, the substances have to be taken up by the epithelial cells either directly by transporters in the membrane or by pinocytosis, i.e., by endozytosis (receptor mediated, constitutive or potocytosis). Then, eventually after modification by the epithelial cell the substances are secreted on the opposing side. This can be done by pumps (ATPases) or transporters, dependent on the electrochemical gradient, if the molecules are free in the cytosol. In case the substances are in vesicles, the release is performed by exocytosis. An uptake performed by vesicles (potocytosis or endocytosis), i.e., by uptake of caveolae or other vesicles, followed by transcellular transport of these vesicles, and fusion to the membrane of the opposing side, is called transcytosis. Besides the transcellular route, many substances are transported through the intercellular cleft (IC) at the lateral boarders of the cells. The IC has to be sealed tight enough in order to avoid uncontrolled transport. For example, in the intestine undigested proteins or carbohydrates must not leave the intestinal lumen in significant amounts as they would act as antigens in the body. On the other hand, ions and water molecules shall be transported through the IC in a controlled fashion. Additionally, the epithelium undergoes continuous and rather rapid self-renewal while maintaining tight cell-cell adhesion. Enterocytes for example are derived from stem cells located in the crypts of Lieberkühn and differentiate as they migrate within three to five days toward the tip of the villus region. During this directional cell migration, the epithelial integrity of the intestine has to be ensured. This requires the adhesive contacts to rearrange and adapt to different conditions in a highly dynamic way. Thus, the diverse functions of the intestinal epithelium require multiple and differentially regulated adhesive systems.

The important biological and medical aspects of adhesion between adjoined cells are well established. In the simple epithelial lining, which can be found for example in the intestine or the renal tubuli, adhesion between cells is mainly accomplished by the so called junctional complex. This junctional complex consists of tight junctions (TJ, zonula occludens), adherens junctions (AJ, zonula adherens) and desmosomes (macula adherens). Besides the junctional complex, gap junctions allow for a molecular exchange between neighboring cells in some tissues. The TJs are a branching network of sealing strands, where each strand is formed from a row of transmembrane proteins. These proteins are mainly claudins and occludins of both cell membranes with their extracellular domains joining directly. The TJ are responsible for sealing the intercellular cleft and for a selective paracellular transport of water or small dissolved molecules.

The AJs, which are mainly composed of cadherins, are responsible for the mechanical strength of the junctional complex. Moreover, desmosomes are also responsible for mechanical strength, forming spot-like interaction sites randomly arranged on the lateral sides of plasma membranes composed of a specialized form of cadherins, namely desmocadherins.
Cadherins are Ca\textsuperscript{2+}-dependent type I membrane proteins, i.e., they are single membrane spanning with the N-terminus localized extracellularly.\textsuperscript{18,27-29} Cadherins are glycoproteins interacting with cadherins of adjoined cells in a mainly homophilic way. These adhesion molecules play an active role in tissue morphogenesis and patterning\textsuperscript{27,28,30} as they are often expressed in a highly tissue-specific manner and found to be important for cell adhesion within a tissue, and therefore the integrity of that tissue.\textsuperscript{31,32} The extracellular part of cadherins generally consists of multiple repeats of a ~110 amino acid module, termed a cadherin ecto-domain (EC), that contains several cadherin-specific motifs.\textsuperscript{33-35} In the past the cadherin superfamily was subdivided into different subfamilies in different ways. This was typically done on the basis of sequence comparisons of the first cadherin domain EC1, on the number of EC-domains and on the functional properties of the cytoplasmic domain.\textsuperscript{34-36} Dependent on the textbook or review one might find different subdivisions into families, thus, the following categorisation is only one of many possibilities. Type I or “classical” cadherins like E- or N-cadherin consist of 5 EC-domains which exhibit a highly conserved \(\beta\)-barrel conformation and contain four Ca\textsuperscript{2+}-binding motifs. The large cytoplasmic domain consisting of 150 to 160 amino acids binds to armadillo proteins and thereby interacts with the actin cytoskeleton. In the first extracellular domain (EC1) classical cadherins are harboring a HAV-motif in the cell adhesion recognition site. Type II or “arylpal” cadherins like VE-cadherin exhibit a highly similar structure like type I-cadherins but the HAV-motif is replaced by other sequences. The desmocadherins like desmocollins and desmogleins resemble the structure of classical cadherins in the extracellular part but the cytosolic portion is different, allowing these cadherins to interact with intermediate filaments instead of actin filaments. The flamingo cadherins and the protocadherins are distinguished from classical cadherins by additional EC-domains. This homology could be verified by a domain-specific protein sequence analysis comparing every LI-cadherin repeat to classical cadherins.\textsuperscript{44,48} Moreover, classical cadherins possess a conserved tryptophan at position 2 in the EC1.\textsuperscript{18} In LI-cadherin a tryptophan is found at the corresponding position within EC3. In EC1 there is a phenylalanine at position 2 which might adopt a similar function as tryptophan.\textsuperscript{37,44,48,52} LI- and classical cadherins were excessively compared at the genomic level in the past. The LI-cadherin gene spans 59 kb, contains 18 exons and harbors large introns, especially in the 5\textprime{} region.\textsuperscript{48} The intron-exon organization is perfectly conserved between repeats 3–7 of LI-cadherin and 1–5 of classical cadherins. Moreover, the genomic structure of the repeats 1–2 and 3–4 is identical for LI-cadherin and highly similar to that of the repeats 1–2 of classical cadherins.\textsuperscript{48} To clarify the genetic and phylogenetic origin of LI-cadherin and the second 7D-cadherin Ksp-cadherin, it might be necessary to investigate the orthologous genes and proteins in other species. Recently, the \textit{cdh17} was investigated in zebrafish kidney development.\textsuperscript{51} \textit{cdh17} is the orthologous gene of the mammalian \textit{CDH17}. However, sequence comparison and a detailed analysis of the expression pattern of the \textit{cdh17}-protein indicated that zebrafish \textit{cdh17} is derived from a gene ancestral to both \textit{CDH16} and \textit{CDH17}. Zebrafish \textit{cdh17} expression pattern comprises a combination of both mammalian cadherins, because \textit{cdh17} is expressed in both kidney and liver/intestinal tissues. It is tempting to assume that subsequent to divergence of the mammalian lineage, a gene duplication took place resulting in the closely related \textit{CDH16} and \textit{CDH17} genes. Till now, no zebrafish ortholog of the mammalian \textit{CDH16} was found.
Physiological and Pathophysiological Distribution of LI-Cadherin

As explained above, LI-cadherin was initially discovered in rat liver and intestine and thus named LI-cadherin. Under physiological conditions, in human and mice, LI-cadherin is exclusively expressed in the small and large intestine but missing in the upper gastric tract and also in the liver. Here and in the following when using the term expression it is meant that the protein can be detected with standard techniques. In many cases where LI-cadherin is not expressed in a specific tissue it cannot be completely ruled out that the mRNA and even the protein are produced but then degraded in a very fast way.

In zebrafish, as explained above, the LI-cadherin ortholog is also expressed in the kidney. The species-specific expression pattern and its biological relevance is still unresolved.

During mouse embryogenesis LI-cadherin expression begins at embryonic day 12.5 as shown by northern- and western-blot analysis. A transient expression was found in the urogenital sinus and the common bile duct on day 13.5. Furthermore, under physiological conditions LI-cadherin was found exclusively in the intestinal epithelium. The expression onset coincides with the formation of intestinal villi. This developmental stage is characterized by excessive tissue remodeling, growth, and differentiation. It should be emphasized that LI-cadherin was found to be always coexpressed with E-cadherin in polarized cells.

LI-cadherin is uniformly distributed along the lateral cell membranes but is excluded from adherens junctions or desmosomes, whereas the coexpressed classical cadherins or desmoscadherins are concentrated in these specialized membrane regions. This was investigated by immuno-gold electron microscopy of rat tissue revealing that LI-cadherin is located solely on the basolateral surface of hepatocytes, enterocytes and goblet cells but is absent on the apical plasma membrane.

Under pathological conditions the distribution of LI-cadherin expression can be changed. Especially in the case of metaplasia and tumor development the LI-cadherin expression is impaired. This is in principal concordance with the function of other cadherins which inhibit tumor growth. Reduced cadherin mediated adhesion is one of the factors that may induce metastasis of cancer cells. Reduced expression or adhesive activity of classical cadherins can be a marker of breast, prostate, colon, and stomach cancer cells. The observations for LI-cadherin are not that simple to interpret. A study of human colorectal cancer cells showed that reduced LI-cadherin expression is associated with high tumor grade, lymphatic invasion and lymph node metastasis. Thus analysis of reduced LI-cadherin expression may help to indicate the biological aggressiveness of malignancy of this tumor. Also in pancreatic tumors LI-cadherin expression can be impaired. In some tumors LI-cadherin is expressed in significant amounts although under physiological conditions LI-cadherin is not found in pancreas cells. LI-cadherin is also found to be upregulated in Barrett's carcinogenesis and Barrett's metaplasia of the esophagus, gastric cancer and intestinal metaplasia. Changed LI-cadherin expressions are found as well in intrahepatic cholangiocarcinoma and hepatic carcinoma. It is presently not understood if the upregulation of LI-cadherin respectively its expression in cells normally lacking LI-cadherin is a cause for the tumor progression or if LI-cadherin is upregulated to compensate e.g., for the impaired E-cadherin mediated adhesion in the corresponding tumor-cells.

Biophysical Properties of LI-Cadherin

LI-cadherin leads to cell aggregation. Soon after the identification of LI-cadherin it was found that LI-cadherin is a functional cell-adhesion molecule. This was mainly found out by cell-adhesion assays. Especially the hanging drop assay proved to be highly valuable. For this cells expressing the cadherin under investigation are trypsinized and resuspended in culture medium. Droplets of the cell suspension are then placed on the inner side of an Petri dish lid. After defined time steps the number of particles in the hanging drop are counted. Counting is done typically some minutes (≥ number of particles N0) and several hours (≥ number of particles Nt) after droplet formation. The aggregation index is then calculated as (N0 - Nt)/N0.

In quantitative cell-cell adhesion assays based on stable transfected CHO cells or S2-cells, LI-cadherin and E-cadherin exhibited almost identical aggregation indices. However, while E-cadherin induced a tight, spherical packing of the cells in hanging-drop experiments and a typical epithelial phenotype of plated cells, LI-cadherin expression resulted in less densely packed clusters in the hanging drop and an intermediate phenotype in the initially fibroblastoid growing CHO cells. LI-cadherin function is obviously sufficient to induce cell clustering at a comparable level to classical cadherins, but the missing link to the cytoskeleton seems to contradict a tight binding and the induction of a fully epithelial phenotype.

LI-cadherin molecules trans-interact directly and specifically. The homotypic, Ca²⁺-dependent LI-cadherin binding was further shown by single molecule atomic force microscopy (AFM), affinity shift chromatography and laser tweezer studies. These studies were mainly performed using a chimeric cadherin construct consisting of the full extracellular domain of the cadherin under investigation fused to the Fc-portion of an IgG (cadherin-Fc). The Fc-part was fused to the C-terminus of the LI-cadherin leaving the N-terminus free. This soluble protein can be purified and used for biophysical measurements.

For AFM force spectroscopy cadherin-Fc proteins are covalently attached to the tip of the AFM-cantilever and to the surface of a plate. The tip and plate are brought into interaction and separation by cyclic up and downward movements of the tip at defined frequencies (force-distance cycles). During downward movement (approach) the tip of the cantilever will eventually hit the plate and, during further downward movement will be gradually deflected upwards until the end of the approach. During the following retrace movement the cantilever bends back with the same linear force slope until the tip separates from the plate to reach the unbent neutral position. During further progression of the retrace movement the cantilever will remain in the neutral position if no interaction between tip- and plate-bound cadherins occurs. Interaction between cadherins can proceed throughout
the entire period during which plate and tip are in contact. If trans-interaction has taken place the cantilever will be pulled down below the neutral line, until a critical force is reached (unbinding force) at which the cadherin bond break. This unbinding event is characterized by an abrupt jump of the cantilever to the neutral position which can be detected. As the cadherins are attached to the plate and tip by flexible PEG-linkers, molecules can freely diffuse within the radius of the length of the linkers (~8 nm) allowing them to undergo unimpaired encounter reactions.\textsuperscript{75,76} Depending on the force loading rate (pulling velocity) the observed unbinding force changes and thus allows to determine the lifetime of the cadherin-cadherin bond. Trans-interacting LI-cadherin molecules exhibit rather weak unbinding forces of 17 to 51 pN at retrace velocities ranging from 150 to 3,000 nm/s and a short lifetime of the LI-LI-bond of 1.41 sec. These findings are in line with those reported for classical cadherins like VE-cadherin (unbinding forces of 35 to 55 pN at 200 to 4,000 nm/s) and a lifetime of only 0.55 sec.\textsuperscript{77-79} The unbinding force and lifetime of N-cadherin (≈40 pN, 1.1 s)\textsuperscript{80} determined with the same approach are in the same order of magnitude. Similar values were determined on living cells for the unbinding forces and lifetimes of VE-cadherin (50 pN; 2.2 s), N-cadherin (30 pN; 1.0 s) and E-cadherin (73 pN; 0.9 s).\textsuperscript{79-81}

Affinity shift chromatography allowed to determine the dissociation constant $K_D$ of the homophilic interaction of LI-cadherin molecules yielded an average $K_D$ of about 27 $\mu$M.\textsuperscript{82} Using this approach the homotypic VE-cadherin binding exhibited an average $K_D$ of 78 (± 21) $\mu$M.\textsuperscript{78}

Specific trans-interaction of LI-cadherin in a cellular context was additionally shown using a laser tweezer approach.\textsuperscript{82,83} Polystyren micro-spheres were coated with a LI-cadherin-Fc and these spheres were allowed to settle onto LI-cadherin expressing CHO-cells. Within 15 min tight adhesive contacts were formed between the cells and the spheres. These contacts were probed by a laser tweezer. The percentage of spheres resisting displacement by the laser can be taken as a measure for the total binding activity.

Besides characterization of the binding parameters of the homophilic interaction of LI-cadherin molecules, the techniques described above also allow the identification of the heterotypic interaction of LI-cadherin and E-cadherin. The heterotypic trans-interaction of LI- and E-cadherin exhibits unbinding forces, dissociation constants, and lifetimes similar to those found for the homotypic trans-interaction of LI- and E-cadherin respectively.\textsuperscript{82} The interaction of LI-Fc-coated micro spheres with E-cadherin-expressing CHO cells was not only associated with the accumulation of E-cadherin at the cell-sphere contact sites but also resulted in a cytoplasmic recruitment of catenins to the contact area. The recruitment indicates that the binding of cellular E-cadherin to micro-sphere bound LI-cadherin induces a cytoplasmic assembly of catenins similar to the homotypic E-cadherin interaction.

**LI-cadherin is localized in cholesterol rich membrane fractions and stays mobile in the plasma membrane.** The short cytosolic portion of LI-cadherin exhibits no structural similarity to the cytosolic domains of classical cadherins. Especially no catenin binding sites can be identified. It is generally believed that LI-cadherin does not interact with the cytoskeleton.\textsuperscript{52} This is in line with recent measurements of the lateral mobility of cadherin molecules in the cell membrane.\textsuperscript{73} The lateral mobility was quantified by FRAP (fluorescence recovery after photo bleaching). For this technique LI-cadherin was fluorescently labeled with yellow-fluorescent-protein (LI-YFP). This chimeric protein was expressed in CHO-cells. Then the YFP was photo-bleached in a restricted area using a laser beam. The time course of the recovery of the fluorescence signal is observed. From the time course of the recovery of the fluorescence signal the diffusion coefficient can be determined. In a recent study LI-cadherin exhibited a diffusion coefficient of 0.124 ± 0.004 m²/s at the contact site in the presence of physiological Ca\textsuperscript{2+}. Upon Ca\textsuperscript{2+}-removal by EGTA the diffusion coefficient rose about 3-fold to 0.420 ± 0.033 m²/s. The authors of the corresponding study concluded that LI-cadherin constitutively forms dimers that freely diffuse in the plasma membrane due to the lack of cytoplasmic interactions. The significantly higher affinity of the homotypic LI-cadherin interaction compared with that of a classical cadherin (see above) is of particular interest in this context. So far it is believed that LI-cadherin does not interact via catenins to the cytoskeleton. Model calculations have shown that both, transmembrane binding to the cytoskeleton and lateral clustering of membrane diffusible adhesion molecules, enhance cell-cell adhesion.\textsuperscript{37} Clustering could thus contribute in addition to the two to three times higher homotypic affinity of the ectodomain to the overall adhesiveness of LI-cadherin. In the case of the chimeric LI-cadherin molecules fused to a GPI-anchor clustering is very likely, since GPI-anchored proteins are associated with raft-like membrane microdomains. An interesting result of a recent study is that in contrast to the E-cadherin, LI-cadherin is exclusively localized in cholesterol-rich microdomains.\textsuperscript{83} Furthermore, the homotypic LI-cadherin interaction is strictly dependent on cholesterol levels. It is yet unclear which kind of cholesterol-rich microdomains (caveolae, rafts, etc.) attracts LI-cadherin. These findings appear to be important with regard to the cellular distribution of LI- and E-cadherin in the intestinal epithelium. As described above LI-cadherin is evenly distributed along the basolateral surface of enterocytes,\textsuperscript{37} the majority of E-cadherin is concentrated in the adherens junctions.\textsuperscript{84} As the regulation of the lateral mobility e.g., by cytoskeletal tethering is important for controlling cellular adhesion,\textsuperscript{76,79,80} preformed cadherin clusters could enhance trans-interactions. This preclustering in combination with the high mobility might give rise to a fast establishment of flexible, i.e., highly dynamic adhesive contacts.

**LI-cadherin trans-interaction is Ca\textsuperscript{2+}-dependent in a highly cooperative way.** The laser tweezer technique as well as the single molecule force spectroscopy using the AFM described above allowed to determine the Ca\textsuperscript{2+}-dependency of the LI-cadherin trans-interaction. The binding activity observed by these two techniques was determined in dependence on the Ca\textsuperscript{2+}-concentration. Trans-interaction of the complete LI-cadherin ectodomain occurred at an apparent $K_D$ of 680 $\mu$M Ca\textsuperscript{2+} in the AFM-experiments and at a $K_D$ of 720 $\mu$M Ca\textsuperscript{2+} in the laser tweezer experiments.\textsuperscript{83} In both cases, Ca\textsuperscript{2+}-binding showed a very high degree of cooperativity with a Hill coefficient $n_H$ of about
12 corresponding to an all-or-nothing response of LI-cadherin-mediated cell adhesion in response to small changes of extracellular $\text{Ca}^{2+}$ in the range of 600 to 800 mM. Those values are close to the physiological free $\text{Ca}^{2+}$-concentration of about 1.2 mM. Thus, LI-cadherin appears to act as a $\text{Ca}^{2+}$-dependent adhesion switch in the lateral contact zones of intestinal epithelial cells.

Calcium ions generally play a crucial role for cadherin structure and function. While it is clear that $\text{Ca}^{2+}$ is vital for cadherin trans-interaction, the role of $\text{Ca}^{2+}$ for cis-dimerization of classical cadherins is under debate. Some studies indicate a $\text{Ca}^{2+}$-independent cis-dimerization\(^{85,86}\) whereas other experiments suggest $\text{Ca}^{2+}$ to be necessary for cis-dimerization.\(^{87,88}\) For LI-cadherin it was recently shown by FRET-experiments that LI-cadherin cis-dimerizes constitutively on the cell surface.\(^{71}\) The observed FRET efficiency found for LI-CFP/LI-YFP within and outside of cell-cell contacts as well as in the presence and absence of $\text{Ca}^{2+}$ was constant. Thus, while $\text{Ca}^{2+}$ is necessary for trans-interaction of LI-cadherin molecules, cis-dimerization of LI-cadherin is clearly independent of $\text{Ca}^{2+}$ as well as of trans-interactions.

**Possible Physiological Functions of LI-Cadherin**

The exact biological function of LI-cadherin is currently not known. It has been speculated that due to the high lateral mobility and the flexible preformed clusters in combination with the unusual low $K_{d}$, LI-cadherin could be responsible for fast and flexible initial formation of adhesive contacts between cells in development and during tissue regeneration.\(^{44,72,73}\) Due to the heterophilic interaction with E-cadherin it might also regulate the E-cadherin mediated adhesion. The non-uniform findings from pathological observations in which up as well as downregulation of LI-cadherin expression might be correlated with tumor generation and metastasis might be in line with this assumption. However, it is presently not clear if the changed expression of LI-cadherin in pathological cases like carcinogenesis is causally involved or if this is a secondary phenomenon.

It is worth noting that the 7D-cadherins are expressed in epithelia which are involved in water resorption under various osmotic conditions. In the gut for example, where LI-cadherin is expressed, water has to be reabsorbed from the chymus to avoid water loss. The luminal content of the gut shows osmolarities from almost pure water to the high osmolarity of the faeces, far above the physiological osmolarity of the interstitium of about 300 mM.\(^{4}\) A similar situation can be found in the kidney where the urine is produced and where KSP can be found in the epithelium of the tubules. It is tempting to assume the involvement of 7D-cadherins in the regulation of water absorption, as in all these organs water transport plays a major role. It was speculated in the past that LI-cadherin might be involved in regulating the width of the intercellular cleft and thereby influencing the water transport.\(^{44,72,89}\)

Another noteworthy fact is the unusual $\text{Ca}^{2+}$-dependency of LI-cadherin binding.\(^{72}\) As we could show recently, LI-cadherin mediated adhesion becomes insufficient at $\text{Ca}^{2+}$-levels just below the physiological concentration of about 1.5 mM. This is in contrast to classical cadherins which can tolerate $\text{Ca}^{2+}$-levels down to 0.3 mM.\(^{72,79,80,90}\)

The facts that (1) 7D-cadherins are expressed in epithelia where water transport under different osmotic conditions takes place and (2) LI-Cadherin displays an extreme sensitivity toward decreased $\text{Ca}^{2+}$-levels led us to the development of a model for the water resorption in epithelia.\(^{89}\) Taking into account that during water transport (due to viscous friction) a small pressure gradient will be built up in the intercellular cleft (IC), the width of the IC $b$ becomes important. In the case of hypotonic medium in the lumen of the resorbing organ (e.g., the gut), a wide cleft (large $b$) facilitates water transport because of friction minimisation. On the other hand, if the medium is hypertonic, a narrow intercellular cleft favors water resorption since in the small volume, an osmotic gradient between the lumen and the IC can be built up by ATPases. This allows for water uptake from the lumen even if the content, e.g., the faeces, exhibits osmolarity far above the isotonic electrolyte concentration. The derived simple theoretical model shows interesting effects in support of the above hypothesis and suggests a role for 7D-cadherins in the regulation of osmotically driven water transport. Changing the width of the intercellular cleft (IC) between neighboring epithelial cells can regulate the direction and efficiency of water transport through a simple epithelium. In a narrow cleft the cells can increase the concentration of osmotic active substances easily by active transport, but the friction of the transported water is high. If the cleft is wide, friction is reduced but the cells can hardly build up high osmotic gradients. As the $\text{Ca}^{2+}$-concentration is principally coupled to the overall electrolyte concentration, the activity of 7D-cadherins is presumably strictly coupled to the osmotic conditions in the water absorbing organs. Clearly the width has a dramatic effect on the electrolyte concentration and on the water flux. As expected for hypotonic conditions in the lumen, a wide intercellular cleft ($b = 400 \text{nm}$) leads to a higher water flux compared with a narrow cleft ($b = 40 \text{nm}$). In the case of a wide cleft the friction is reduced and the osmotic gradient can be maintained by diffusion of the electrolyte from the interstitium into the cleft.\(^{89}\) The concentration in the IC follows very much the luminal concentration. However, under hypertonic conditions the water flux is inverted, i.e., water flows from the interstitium into the lumen if the cleft is 400 nm wide. Notably this is not the case if the cleft is narrow. For $b = 40 \text{nm}$ the volume of the intercellular cleft is small, leading to a concentration in the IC significantly higher than the luminal concentration due to the electrolyte flux maintained by the ATPases. Under these conditions the osmotic gradient is still directed from the lumen into the cleft allowing to further increase the osmolarity of the luminal content.

Thus, it is tempting to assume that active 7D-cadherins, due to their trans-interaction with cadherins of neighboring cells, will cause a narrowing of the intercellular cleft. 7D-cadherins due to their location and their $\text{Ca}^{2+}$-dependence could thus provide a way to passively adapt the direction and efficiency of water transport through epithelia. It has to be emphasized that no experimental proof for this hypothesis could be given so far. Thus, the exact physiological role of LI-cadherin remains enigmatic.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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