Tight Junctions and Paracellular Fluid and Ion Transport in Salivary Glands

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Primary saliva is formed by salivary epithelial endpieces through two pathways, the transcellular and the paracellular pathways. While the mechanisms of ion transport through the transcellular pathway have been well studied, our understanding of fluid and electrolyte transport through the paracellular pathway remains rudimentary. Increasing evidence indicates that the tight junction (TJ) proteins form and regulate the paracellular pathway, although other intercellular junctions are probably involved. The structure of the TJ is complex and has not been well characterised. A functioning TJ is formed by multiple proteins, including membrane, cytoplasmic scaffolding, and signalling proteins. Paracellular fluid and electrolyte flow is mediated by high-capacity, charge- and size-restrictive small pores with a radius of 4 to 6 Å, whereas macromolecules pass through low-capacity, nonrestrictive large pores. Although the characteristics of these pores need to be further delineated, it is clear that they are under the regulation of the autonomic nervous system, endocrine, paracrine and autocrine systems, and various pathological factors. To date, the majority of the evidence for paracellular fluid and ion transport is accumulated from the studies using various epithelia other than salivary glands. Further investigations to explore the structure, function, and regulation of the paracellular pathway in salivary epithelia are needed to better understand the mechanism of saliva secretion.

Key words: *tight junction, paracellular fluid and ion transport, saliva secretion*

Introduction

It is well recognised that saliva secretion is a two-stage process, as first proposed by Thaysen et al[1] in 1954, who suggested that the endpieces of salivary glands secrete a plasma-like isotonic fluid, called primary saliva. Modification of the primary saliva occurs, as it passes through the duct system, with the composition altered by a number of ion transport mechanisms, resulting in a hypotonic fluid as it reaches the oral cavity. This hypothesis was confirmed in 1966 when Martinez et al[2], utilising a micropuncture technique, collected primary saliva from the intercalated duct and measured its composition. This well-designed experiment clearly demonstrated that primary saliva is isotonic. In the same year, Mangos et al[3] and Young et al[4] also reported similar results using the same technique. Subsequently, the two-stage process of saliva secretion has been verified to include a 'leaky' secretory endpiece (acini), which is highly permeable to water and a 'tight' ductal system with very low water permeability.

Several theoretical models have been proposed describing the mechanisms of transepithelial fluid transport, including the osmotic coupling, electro-osmotic,

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cotransporter, Na^+ recirculation, and osmosensor feedback theories (see review by Hill)[5]. It is well recognised that transepithelial fluid transport is mediated by two major pathways, the transcellular pathway and the paracellular pathway. Fluid secreted through the transcellular pathway passes the basolateral membrane, the cytoplasm, and the apical membrane to enter the lumen, whereas fluid transported via the paracellular pathway enters the lumen from the interstitium by passing through the tight junction (TJ) structure.

Transcellular fluid and ion transport

Since the two-stage secretory process in salivary acini was established, experimental evidence now confirms the underlying mechanisms of fluid transport in acini. The widely accepted model for salivary acinar cell secretion[6-9] (Fig 1a) is based on the 1977 hypothesis proposed by Silva et al[10] for the shark rectal gland, which has been used to describe fluid secretion in a variety of epithelia, including salivary glands. According to this model, Na^+-K^+ pump in the basolateral membrane of acinar cells transports 3 Na^+ ions from within the cell and concentrates 2 K^+ ions into the cytoplasm. This inwardly directed Na^+ gradient established by Na^+-K^+ pump in turn activates a Na^+-K^+-$2Cl^-$ cotransporter, the so-called 'secondary active transport system', to transport 1 Na^+, 1 K^+, and 2 Cl^- ions into the cell, maintaining the cytosolic Cl^- concentration above its electrochemical equilibrium. This Cl^- gradient across the cell membrane provides the driving force for Cl^- or HCO_3^- ion flow into the lumen through an anion channel in the apical membrane. With α_1 adrenergic or muscarinic cholinergic agonist stimulation, cytosolic free Ca^{2+} concentration is increased, leading to KCl efflux via anion channels in the apical and K^+ channels in the basolateral or lateral membranes. Although K^+ channels in apical acinar membranes are proposed[11], further evidence of ion transport measurements and/or channel characterisation are needed. Luminal Cl^- efflux, creating a net electrical charge and attracting accompanying Na^+ movement through TJs, produces an osmotic gradient across the epithelium, attracting water movement from the cytoplasm via a water channel, most likely aquaporin (AQP) 5. Water may also move from the interstitial space via TJs into the lumen. However, the role of aquaporin channels in transepithelial fluid transport is under intense debate[5]. Additional data exist confirming the role of other ion transporters in the basolateral and lateral membrane, including a Na^+-H^+ exchanger and a Cl^--HCO_3^- exchanger, contributing an important role in the maintenance of cytosolic pH[12,13].

Paracellular fluid and ion transport

Although great progress has been achieved in revealing the characteristics, function, and regulatory mechanisms of transcellular ion transporters, our understanding of paracellular fluid transport in salivary epithelia remains rudimentary. The physiological properties of paracellular fluid transport have not been clearly characterised, although various mathematical models exist, attempting to characterise the system. Since reliable methods are still not available to accurately measure water transport via a specific route, it is not possible to experimentally distinguish the proportion of water movement via a transcellular or paracellular pathway in specific acinar epithelia, including salivary acini. For example, it remains unclear which pathway in the salivary acini, transcellular or paracellular, is the primary mechanism regulating water, Na^+ and other ion movement; what role AQPs play, specifically AQP5, in fluid transport; and what alterations in TJs occur in salivary gland dysfunction, specifically in salivary gland hypofunction found with Sjögren syndrome.

Structure of the TJ complex

There are four types of intercellular junction complexes between epithelial or endothelial cells, TJs, adherens junctions (AJs), desmosomes, and gap junctions. AJs and desmosomes form strong mechanical connections between adjacent cells to link cells together, and gap junctions provide a unique venue for cell-to-cell communication. TJs are protein sealing complexes between cells and form a belt-like structure among neighbouring cells, encircling cells at the apical side of the lateral membrane and occluding the intercellular space to separate the lumen and interstitial space through the function of barrier and fence. The structure of TJs appears like a string of continuous particles inlaid into the membrane, forming TJ strands. One of the major functions of TJ complex is to form a barrier and channels to water, ions, and other small molecules, regulating their passage through the paracellular pathway. TJ strands are formed by TJ proteins, which constitute pores allowing some ions and molecules with specific size or charge to pass through (Fig 1b). The function of the TJ barrier is often characterized by using transepithelial electrical resistance (TER). However, TER reflects the contribution from both transcellular and paracellular electrical resistance, and cannot be used to quantify the specific contribution of TJ barrier to the resistance. In addition, TER often does not correlate with permeability and density of pores, making interpretation more difficult. The second function

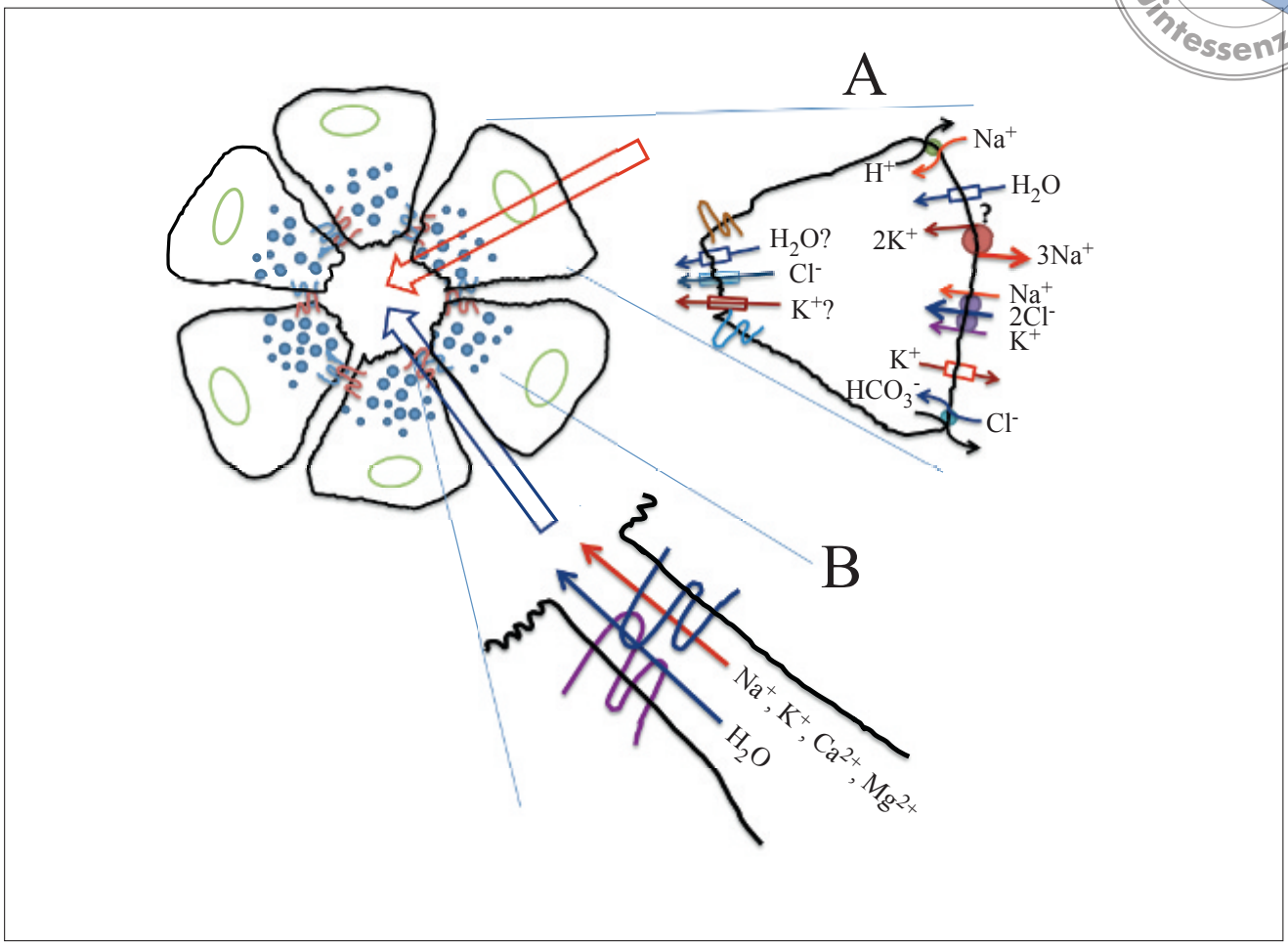

Fig 1 Transepithelial fluid and ion transport model in salivary acini. (a) Transcellular fluid and ion transport model. A transmembrane Na^+ gradient is established by the basolateral Na^+, K^+-pump; the gradient drives Cl^- and K^+ into cells through the Na^+-K^+-$2Cl^-$-cotransporter. Cl^- and HCO_3^- are secreted into the lumen via the apical anion channel. K^+ flows out through a basolateral K^+ channel and probably a hypothetic apical K^+ channel. The pathways for water to flow in and out the cytoplasm remain unclear, assuming that water entry is mediated by a basolateral aquaporin (AQP) and water exit is via AQP5. However, direct evidence showing that AQP5 mediates water flow is lacking. The pH of the cytosol is maintained by two mechanisms, the Na^+-H^+ exchanger and the Cl^--HCO_3^- exchanger, both located at the basolateral membrane. (b) Paracellular fluid and ion transport model. The strands formed by the integral membrane TJ proteins constitute a barrier for water and ions and a fence for macromolecules including peptides, proteins, lipids, and any other large substances. Two types of pores are formed by TJ, small and large pores. Na^+, K^+, probably Ca^{2+} and Mg^{2+}, as well as some other ions are transported through the small pores and macromolecules pass through the large pores. Although water flow may be mediated by paracellular pathway, the major pathway for water flow remains uncertain.

of the TJ complex is to act as a fence to maintain cell polarity by blocking the diffusion of proteins, lipids, and other large molecules between the apical and basolateral spaces. This is typically critical for some epithelia, such as intestinal mucosa, since the fence function plays a vital role in blocking antigens from entering the systemic blood stream circulation and allowing for some nutrients and drugs to be absorbed. A number of detailed comprehensive reviews are available, characterising the structure and function of TJs of specific tissues[14,15], including the salivary glands[16]. Although TJs provide a dynamic sealing among adjacent cells, they are not considered a strong mechanical force linking cells and/or maintaining epithelial structure; the strong linkages between cells are formed by AJs and desmosomes.

TJ strands

TJ strands are the interacting network between cells visualised with electron microscopy, formed by extracellular loops of the transmembrane TJ proteins from one cell membrane paired with the matching loops from the

adjacent cell membrane. Many studies observed that the number of TJ strands and the complexity of the network in an epithelium are correlated with the tightness of the epithelium and is known as the *Claude hypothesis*[17]. This theory has been evident in a variety of epithelia, including salivary acini. For example, Simson and Bank[18] observed that rat parotid acini have an average of 2.5 strands, whereas the striated duct has 6.0 strands. Kikuchi et al[19] found that individual male murine acinar cells of the submandibular gland (SMG) have an average of 3.9 strands, whereas the individual granular duct cell has 10.4 strands. Similarly, in the female murine SMG, individual acinar cell has 4.1 strands, whereas each granular duct cell has 7.4 strands. As previously discussed, salivary acini have a high permeability to water, while the duct system has extremely low permeability. Acini also have high paracellular permeability to Na^+; however, it is unclear if the TJ complex of duct cells is permeable to Na^+. The thickness and complexity of TJ strands can be semi-quantitatively measured under electron microscopy.

TJ depth

TJ depth is an index that has been frequently used to quantify the thickness of the TJ complex. Various studies indicate that TJ depth is inversely correlated with the paracellular permeability of fluid in an epithelium. For example, TJs in mouse SMG acini and intercalated ducts are 0.10 to 0.15 μm in depth, whereas it is 0.5 μm in granular and striated ducts[19]. Similarly, TJ depth is 0.25 μm in rat parotid acinar cells and 0.43 μm in the striated duct[18]. In addition, TJ depth can also reflect the pathological changes in TJ structure. Treatment of salivary glands with proinflammatory cytokines such as tumour necrosis factor (TNF)-α and interferon (IFN)-γ significantly reduced TJ depth and impaired secretory function of the glands[20].

TJ width

Another index of the TJ complex is TJ width, although it is utilised less frequently and its significance remains to be further confirmed. TJ width is defined as the distance between the two inner plasmalemmal leaflets of a TJ shared between adjacent cells[21,22]. It has been used in various tissue types, including rat myenteric plexus capillaries[22], nonhuman primate endometrial microvessels[23], human placental capillaries[24], rat gastric mucosa[21], murine intestinal mucosa[25], and rabbit SMGs[26]. Functional studies indicate that TJ width may be related to barrier function in intestinal mucosa[25]. TJ width and saliva secretion are found to be reduced in transplanted rabbit SMGs and with recovery of structure and function after stimulation with capsaicin[26]. Although TJ width seems to be a sensitive index of TJ function, the mechanism underlying this phenomenon needs to be further investigated since the distance between two adjacent cells is theoretically controlled primarily by AJs and desmosomes, instead of TJs. The so-called 'TJ width' may actually reflect the structure and function of AJs. There has been evidence indicating that TJs and AJs are structurally and functionally linked, despite being formed by distinct proteins[27]. It has been observed that AJ proteins are involved in regulating fluid, electrolyte, and macromolecule transport in epithelia, including salivary acini[26,28]. This is an interesting and important, but virtually underdeveloped area in paracellular fluid and ion transport in salivary glands.

TJ proteins

The TJ complex is formed by TJ proteins. Accumulating evidence indicates that some dysfunction of TJ proteins may underlie the pathogenesis of a variety of clinical disorders, such as autoimmune diseases, cancer, infectious diseases, and allergy[29]. For example, emerging data indicates that TNF-α and IFN-γ alter TJ structure and function[20]. Cytokines-induced TJ dysfunction is one of the major factors leading to the salivary gland destruction in Sjögren syndrome[30].

Functioning TJs consist of three types of protein components: transmembrane, cytosolic scaffolding, and cytosolic signalling.

Transmembrane proteins

TJ strands are formed by transmembrane proteins; this category has a large number of integral membrane proteins, including a set of tissue- and cell-specific claudins, the TJ-associated MARVEL protein (TAMP) family, and the junction adhesion molecules (JAMs).

Claudins

The first claudin was discovered in 1998[31] and 27 claudin proteins have been identified[32], each having a molecular weight of 20~27 kDa. The structure of claudins includes four transmembrane domains, two extracellular domains, and both N-terminus and C-terminus within the cell. The extracellular domains constitute two loops. The larger loop has 49 to 52 residues and the smaller loop 16 to 33 residues. Strong evidence indicates that the large loop and the matching loop of another claudin or occludin in an adjacent cell form one type of TJ pores with size and charge selectivity[14]. However, each claudin may not have

the same charge-discriminative capability, depending on the sequence alignment of the loop. The difference of barrier property between cell types is probably due to the variation in combination of claudins[33]. A frequently referred example is the larger loop of claudin-2, which has more negative charge in the amino acid residues; therefore, when expressed in testing cell lines such as Madin-Darby canine kidney (MDCK) cells, TER is reduced and Na^+ permeability is increased[34,35]. Claudin-2 is often expressed in leaky epithelia, including salivary epithelia, and has therefore been called 'leaky' claudin[34,36]. In contrast, claudin-14 has more positive charge in its amino acid loop sequence and increased expression significantly enhances TER and reduces Na^+ permeability. Functional changes similar to the claudin-14-like structure have also been observed after overexpression of claudin-4[37], -5[38], and -8[39]. Claudin-2, -10b, -15, and -16 form cation-selective pores[14]. Most TJ pores usually have a strong preference for Na^+ versus Cl^- ions, except from the pores formed by claudin-14. This has critical significance in salivary acinar ion transport since it allows Na^+ flow across the paracellular barrier to follow negative charge (the electro-osmotic gradient) established by anion (Cl^- or HCO_3^-) secretion via the transcellular pathway, specifically, the apical anion channel. The higher resistance to Cl^- also prevents Cl^- backflow to the interlateral or interstitial space. To date, the accurate mechanisms of pore formation by claudins remain unclear. This will be an important research area in elucidating the relationship between claudins and ion- and size-selectivity of the paracellular pathway.

The function of claudins can be elucidated at the animal level by using claudin-knockout or -knockdown animal models. There have been a number of claudin-deficient mouse models, revealing the morphological and functional alterations or impairments induced by the absence of certain claudins (Table 1). An additional method to detect the function of claudins is to express a specific claudin in deficient cell lines or to overexpress or knockout the claudin in cell lines that naturally express the protein. In the last 15 years, many studies have explored the role of claudins in paracellular fluid and ion transport, and the relationship between claudin types and ion selectivity is becoming clearer.

TAMPs

The TAMP family includes MarvelD1 (occludin), MarvelD2 (tricellulin), and MarvelD3 proteins. Similar to claudins, all three TAMP members have four transmembrane domains and two extracellular loops. Human occludin is encoded on chromosome 5q13.1 and has 522 amino acids. Although occludin often participates in forming TJ strands[57], its sequence is not similar to claudins[31]. It is unclear whether the TJ strands formed by occludin have the same function as the strands formed by claudins. Overexpression of occludin induced formation of intracellular multilamellar bodies[58]. It also has been observed that inclusion of occludin in TJ strands can increase both TER and paracellular permeability[59,60]. Many *in vitro* and *in vivo* studies reported that occludin plays an important role in intercellular adhesive interactions and TJ barrier function[61-64], although other studies indicated that occludin appeared unessential to TJ function since the lack of occludin did not impact TJ structure and function in endothelial and Sertoli cells[65,66]. However, additional studies clearly showed that occludin knockdown induced diverse phenotypic changes in epithelial cells[62]. Mice lacking occludin exhibited many pathological signs, including growth retardation, testicular atrophy, male infertility, brain calcification, compact bone thinning, and gastric inflammation[67,68]. Salivary glands were also involved; the morphological changes included loss of cytoplasmic granules in striated duct cells and glands were dysfunctional[67,68]. Occludin appears to have some role in directing tricellulin expression. Knockdown of occludin in MDCK II cells induced tricellulin to be mislocalised to bicellular contacts[69]. The mislocalisation increased TER and permeability to ions and large solutes[70]. Occludin also plays a critical role in the capsaicin-stimulated TER decrease as well as trypan blue and fluorescein isothiocyanate (FITC)-labeled dextran flux in rat SMG acinar cell line SMG-C6 monolayers, since the TER response to capsaicin was suppressed by occludin knockdown with shRNA and restored by occludin re-expression[71].

MarvelD2 is also called tricellulin, which is expressed at tricellular contact locations[72] (see review by Mariano et al[73]). The strands of tricellular TJs constitute a tubular structure with a 10-nm diameter (the central tube) that is vertical to the bicellular TJ belt[73]. The potential function of the central tube may be to control macromolecule flow[70]. Tricellulin has 555 amino acids with a molecular weight (MW) of 63.6 kDa and can form a homomeric complex[74]. However, studies have reported discordant results regarding the formation of heteromeric complex with occludin[74,75]. Tricellulin is also needed for TJ organisation. Knockdown of tricellulin in mouse mammary epithelial (EpH4) cells impaired TJ structure at both bicellular and tricellular contacts[72], reduced TER, increased paracellular permeability[69], and delayed barrier development[75].

MarvelD3 is a new member of the TAMP family; it was identified by Steed et al in 2009[76]. MarvelD3 is a 40-kDa protein and is expressed in many epithelial and

Table 1 Primary characteristics of claudin-deficient mice

Mouse model	Primary characteristics of the model
Claudin-1-deficient mice (Cldn1$^{-/-}$)[40]	Die within 1 day of birth; lack the epidermal barrier; tracers with molecular weight of 600 Da diffuse across TJs easily
Claudin-2-knockout mice[41]	Normal appearance, activity, growth, and behaviour; the kidney is histologically normal, but TJs of the proximal tubules are poorly developed; decreased transepithelial reabsorption of Na^+, Cl^- and water; loss of cation selectivity
Claudin-2-knockout mice[42]	Reduced paracellular Na^+ permeability in the small intestine
Mouse model for muscular dystrophy and cardiomyopathy[43]	Claudin-5 is significantly reduced; expression of claudin-5 prevents the pathological changes of cardiomyopathy
Caludin-6-null mice[44]	No obvious anatomical phenotypic abnormalities
Claudin-7-knockout mice (Cldn7$^{-/-}$)[45]	Born alive and die within 12 days after birth; severe salt wasting, chronic dehydration, and growth retardation with significantly elevated urine Na^+, K^+ excretion and water loss due to lack of transepithelial water barrier
Claudin-7-deficient mice[46]	Severe intestinal defects, including mucosal ulcerations, epithelial cell sloughing, and inflammation; gaps below TJs; increased intestinal production of cytokines, the nuclear factor κBp65 subunit, cyclooxygenase-2, matrix metalloproteinases-3 and -7
Claudin-9-null mice[47-49]	These mice are deaf due to TJ complex dysfunction in inner ear including abnormal ion concentrations in the fluid surrounding the basolateral surface of outer hair cells
Claudin-10-deficient mice[50]	Loss of claudin-10 in the thick ascending limb of Henle's loop, inducing hypermagnesemia and nephrocalcinosis; decreased paracellular Na^+ permeability and increased Ca^{2+} and Mg^{2+} permeability
Calaudin-11-null mice[51]	Lack TJ strands in central nervous system myelin and Sertoli cells
Calaudin-11-null mice[52]	Lack TJs between the base cells; normal K^+ recycling; elevated hearing thresholds
Claudin-14-knockout mice[48]	Deaf with normal endocochlear potential; during the first 3 weeks of life, cochlear outer hair cells are rapidly degenerated, whereas the inner hair cells gradually degenerated
Claudin-15-knockout mice (Cldn15$^{-/-}$)[42,53]	Cldn15$^{-/-}$ is not foetal. These mice have megainstestine; expression of other claudins is not altered; decreased luminal Na+ and reduced glucose absorption in the small intestine
Claudin-16-deficient mice[54]	Hypercalciuria and hypomagnesemia similar to familial hypomagnesaemia with hypercalciuria and nephrocalcinosis, however, without nephrocalcinosis
Claudin-16-knockout mic[55,56]	Defective cation permeability in thick ascending limb, leading to perturbation in salt and acid-base balance; increase in urine, and decrease in HCO_3^- excretion and urine pH

endothelial cells. Functional studies indicate that it is not essential for TJ formation, but lack of expression increased TER in Caco-2 cell monolayers. Raleigh et al[75] characterised marvelD3 protein and found that its function is not essential. Overall, TAMP proteins have redundant functions, but are unable to complement each other functionally.

JAMs

The JAM family has three proteins, JAM-A, -B, and -C (also called JAM-1, -2, and -3) with a molecular weight about 40 kDa. The common structure of JAMs includes a single transmembrane domain with its C-terminus in the cytoplasm and N-terminus outside of the cell. The N-terminus has two immunoglobulin (Ig)-like loops

and the C-terminus has type II PDZ domain-binding motifs (PDZ is an abbreviation of the following term: post synaptic density protein, Drosophila disc large tumour suppresser, and zonula occludens-1 protein). JAMs are widely expressed in endothelial cells of lymphatic tissues, the brain, and a variety of epithelia. JAM-A is expressed in human PG and SMG cells[77], primary cultures of SMGs[78], as well as cell line Par-C10[20] and HSG[79]. Whether JAM-B and -C are also expressed in salivary gland epithelia needs further investigation.

JAM proteins regulate a variety of cell functions, including migration of leukocytes, endothelial and epithelial cells, as well as paracellular permeability. It has been well demonstrated that JAMs participate in forming TJ strands and are critical for TJ barrier and fence functions[80,81]. Since most studies have explored the function of JAM-A, data on JAM-B and -C function are scant. The colonic mucosa from JAM-A-deleted (JAM-A$^{-/-}$) mice had normal epithelial structure, but decreased TER, increased dextran flux, and inflammation. Expression of claudin-10 and -15 was increased in JAM-A$^{-/-}$ mice, indicating JAM-A may participate in regulating expression of these claudins[82]. It is well established that the regulatory function of JAMs is mainly facilitating intracellular interactions with scaffolding proteins through their PDZ domain-binding motifs[83,84].

Coxsackie and adenovirus receptor

The coxsackie and adenovirus Receptor (CAR) is a cell adhesion protein with a molecular weight of 46 kDa. CAR was first reported in 1997 as a common receptor for coxsackie virus group B and adenovirus groups 2 and 5 and plays a role in viral attachment and entry. It has been demonstrated that CAR is essential for embryonic development. Deletion of CAR gene or knockout of CAR in the early stages of the embryos induced haemorrhage and embryonic death[85]. CAR is heavily expressed in the brain and heart during embryonic development and predominately expressed in TJs of epithelial cells in adult mice. In the TJ complex, CAR is co-localised with ZO-1 and occludin and is correlated inversely with paracellular permeability[86]. Knockdown of CAR in Sertoli cells caused decreased TER and increased endocytosis of occludin. In contrast, overexpression of CAR increased TER[87]. It is unknown whether salivary epithelia express CAR and what roles CAR plays in paracellular fluid and ion transport under physiological and pathological conditions.

Lipolysis-stimulated lipoprotein receptor

The lipolysis-stimulated lipoprotein receptor (LSR) is a recently identified transmembrane protein, situated between three adjacent cells, and its role is a definer of cell contacts of tricellular TJs (tTJs) in epithelial cells[88]. LSR is a 65-kDa protein with a single transmembrane domain and an Ig-like extracellular domain. The function of LSR is not completely clear. Masuda et al[88] observed that LSR may play a part in three aspects:

- Regulating occludin distribution: it has been observed that occludin distribution in LSR-knockdown EpH4 cells was disturbed and there was an abnormal accumulation of occludin in the tricellular area.
- Regulating TER: LSR-knockdown reduces TER in EpH4 cell monolayers.
- Recruiting tricellulin: LSR interacts with tricellulin. In LSR-knockdown EpH4 cells, tricellulin was not localised at tricellular region, but distributed throughout the basolateral membrane[88].

Studies have not been performed to reveal whether salivary epithelia express LSR and what the physiological role it has in saliva secretion.

Cytosolic scaffolding proteins

The cytoplasmic scaffolding proteins have one or multiple PDZ domains and can bind to various integral membrane proteins such as claudins, occludin and JAMs, as well as actin filaments through different domains, stabilising protein complexes and connecting TJs to the actin cytoskeleton. The number of the PDZ domain-containing proteins is increasing, including membrane-associated guanylate kinase (MAGUK)-like proteins, protein associated with Lin-7 (Pals1), AF-6/afadin, atypical protein kinase C (aPKC), isotype-specific interacting protein (ASIP), partitioning-defective protein-3 (PAR-3), multi-PDZ domain protein 1 (MUPP1), and protein associated with TJs (PATJ). The whole spectrum of functions of these cytoplasmic scaffolding proteins remains incompletely clear, but it is evident that these proteins play a critical role in the organisation and localisation of TJ strands, since blocking PDZ domain induces TJ strands to be poorly organised and distributed to other areas[89].

Zonula occludens proteins

Zonula occludens (ZO) proteins belong to the MAGUK protein family and have three members, ZO-1, -2, and -3. These proteins have multiple important functions, such as interacting with nuclear and dual residency proteins and participating in regulating cell growth and proliferation (see reviews by Bauer et al[90] and Gonzalez-Mariscal et al[91]). ZO-1, -2 and -3 each has a molecular weight of 220 kDa, 160 kDa, and 130 kDa, respect-

ively. Each ZO protein has three PDZ domains, one Src homology 3 (SH3) domain, one guanylate kinase (GU) domain, and a proline-rich region. There are also several unique (U) domains in each ZO protein molecule, localised between other domains. Among them, U5 is localised between SH3 and GUK to form a region of SH3-U5-GUK-U6, which has been demonstrated to play a critical role in TJ assembly and localisation. ZO proteins can associate with several proteins through PDZ domains, such as with JAM-A, claudins, occludin, and F-actin. For example, ZO-1 binds to claudin, ZO-2, and JAM-A through its PDZ-1, -2, and -3 domains, respectively, interacts with occludin through its GK domain, and binds to F-actin simultaneously. Its association with F-actin is through the actin-binding region (ABR). The association with multiple proteins makes it possible to form a large protein complex, linking TJ strands and cytoskeleton. ZO proteins also can associate with signalling proteins, including the serine protein kinase ZAK and G-protein α-subunit. ZO proteins not only bind to TJ proteins, but also associate with AJs and gap junctions[90,91].

ZO-1 and -2 are critical for embryonic development. A knockout of either protein is lethal for mouse embryos, but a lack of ZO-3 did not show a similar response[92]. Silencing or knockdown of ZO-1 or -2 , but not -3 in cultured cell lines exhibits clear changes in TJ function, including decreased TER and increased paracellular permeability to solutes larger than 4 Å[93,94].

Cytosolic scaffolding protein complexes

There are a number of other cytosolic proteins playing critical parts in TJ formation and function, such as forming cytosolic scaffolding protein complexes, among which the Par-3-aPKC-Par-6 complex and the Crumbs-Pals1-PATJ complex play critical roles in TJ formation. The expression and function of these cytosolic scaffolding proteins in salivary glands need to be further explored.

Cytosolic signalling proteins

TJ structure and paracellular barrier function are regulated by many factors through intracellular signalling proteins. This has been a robust research area and a large number of investigations have been performed to delineate the intracellular signalling systems that participate in the regulation process, including protein kinase (PK) A, PKC, Rho kinase, myosin light chain kinase (MLCK), GTPase Rab13, tyrosine kinase (TyrK), and mitogen-activated protein kinase (MAPK). These signalling proteins are not specific TJ proteins, but they are essential factors for the establishment and function of TJs. To date, the major MAPK members, extracellular signal-regulated kinases (ERK)1/2 have been shown to be the potential key factor for the signalling network in various cell types, including salivary cells, although the number of studies utilising salivary cells was limited. A brief discussion of these intracellular signalling proteins is provided in this review and detailed information on the topic will be discussed separately.

In salivary cells, PKA appears to play a role in regulating TJ functions. The Hg^{2+}-induced increase in TJ permeability may be mediated by a mechanism associated with PKA through alterations in the phosphorylation status and distribution of occludin in Par-C6 cells[95]. It has been observed that MAPK may be the major signalling system regulating the TJ function in salivary cells. This includes Src and p38 MAPK, which has been observed to suppress occludin expression in primary cultured rat parotid acinar cells[96,97]. It also has been reported that the Raf-MEK-ERK signal pathway is involved in down regulation of occludin in parotid cell line Pa-4[98]. Most recently, Cong et al[26,71] repeatedly observed that ERK1/2 is the intracellular signalling molecule to mediate the transient receptor potential vanilloid subtype 1 (TRPV1)-activated increase in paracellular tracer permeability, expression of TJ proteins, and internalisation of occludin in rabbit SMGs and rat cell line SMG-C6. Other protein kinase such as PKC and TyrK may play a role in the regulation of TJ remodelling and function, however, their action may be in more upper stream sites. Hamm-Alvarez et al[99] observed that activation of Etk/Bmx, a member of nonreceptor tyrosine kinases, increased TER and disturbed the distribution of actin and occludin in parotid cell line Pa-4. Obviously, more innovative investigations on these issues will provide valuable clue to the TJ structure and function.

TJ formation and remodelling

TJ is a highly dynamic structure and its components undergo continuous remodelling, i.e. assembling and disassembling.

TJ formation

The formation of TJs is a complex process and remains poorly understood. Increasing evidence indicates that AJs are formed first and TJ assembly follows. After AJs are established, the transmembrane AJ protein nectin recruits TJ proteins, including JAM, claudins, and occludin to the apical side of AJs[100]. This process may involve

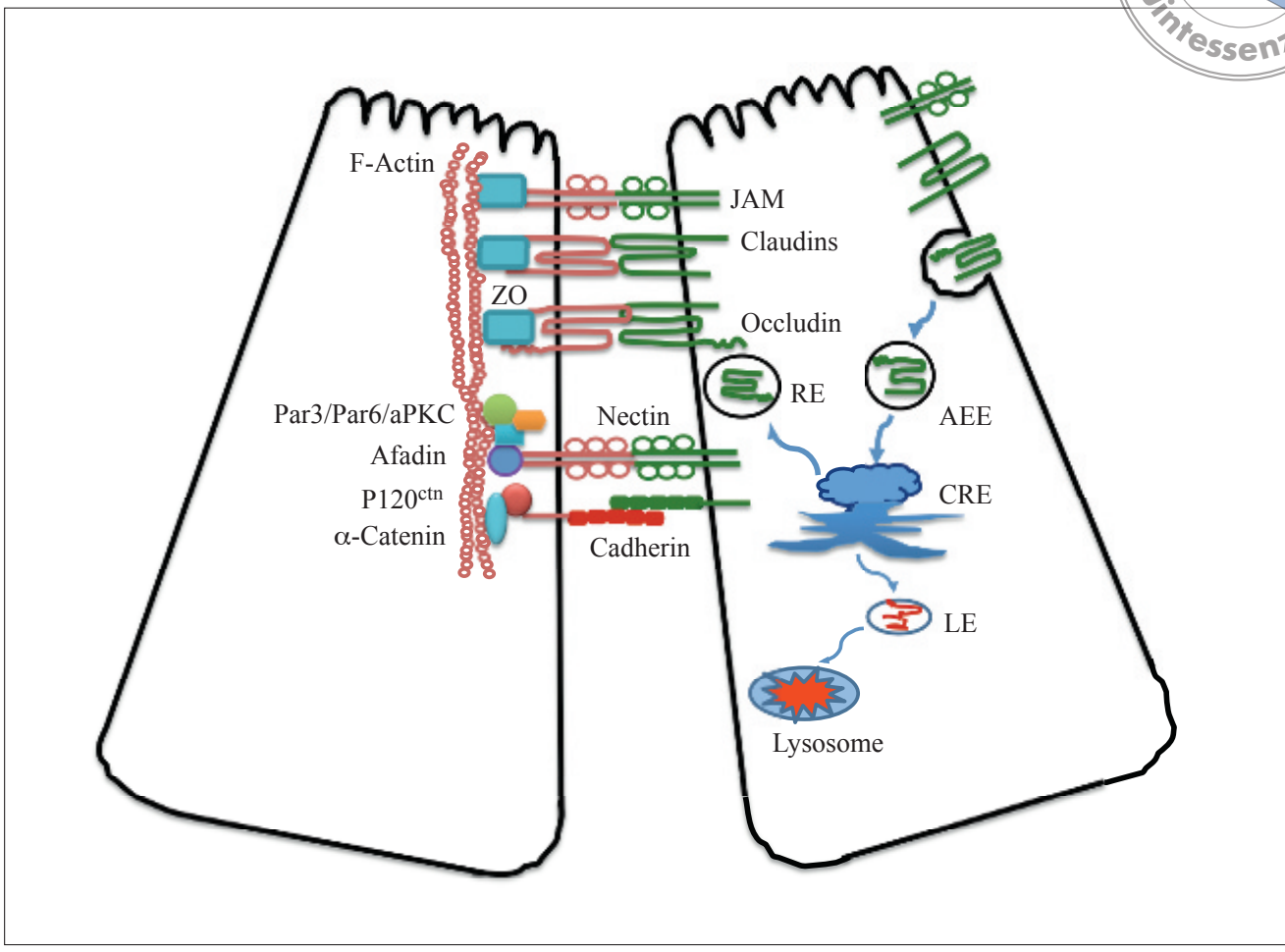

Fig 2 The schematic process of formation and remodelling of TJs. TJs are formed after AJs. AJ protein nectins form first intercellular connection and interact with afadin and the Par-3/Par-6/aPKC complex, recruiting another AJ membrane protein cadherin to establish AJs. Then, TJ integral proteins, JAMs, claudins, and TAMPs, are recruited to the apical site of AJs. All these membrane proteins are connected to F-actin through the cytosolic scaffolding proteins ZO-1, -2, or -3[101]. TJ proteins can be dismantled by one of three types of endocytosis. Internalised proteins are delivered to apical early endosome (AEE), and then the common recycling endosome (CRE) for sorting. Internalised proteins have two final destinations, being reinserted back to TJs through a transport vehicle, the recycling endosome (RE) or being degraded by lysosome through the late endosome (LE)[107]. (The figure is based on figures by Ooshio et al[101] and Ivanov et al[107]).

a number of cytosolic proteins, including cell polarity proteins and signalling proteins. It has been observed that nectins form first cell-cell connection; then the cytosolic tail of nectin interacts with afadin and Par-3 to recruit cadherin to form AJs. Claudins are next to be recruited to the apical side of AJs to generate TJs (Fig 2)[101]. However, the detail of the process still needs to be elucidated.

Structure of TJs

There have been a number of excellent reviews characterising the structure of TJs[16,102-104] and we will provide only a brief summary here. Under an electron microscope, TJs appear as strands. The depth and complexity of the strands reflect the tightness of the epithelia. Some organs, such as the kidney, have different segments of the epithelium, requiring various degree of "tightness" in different segments. The salivary gland epithelium also has leaky endpieces and a tight duct system. In general, the transmembrane proteins are associated with cytosolic scaffolding proteins, including ZO-1, -2, and -3; the latter bind to the cytoskeleton system and signalling proteins, such as protein kinases, phosphatases, and transcription factors (Fig 2).

TJ remodelling

TJs undergo continuous remodelling. TJ disassembly is the major mechanism required for component reorganisation, including removing or reinserting proteins. Claudins, such as claudin-1, may be relatively stable in TJ structure[105], but ZO-1 and occludin are highly mobile. Steed et al[106] estimated that 80% of occludin is diffused in TJs, and a large portion of ZO-1 is undergoing an exchange between TJs and cytosolic pools. However, the conditions and regulatory mechanisms of such dynamic process are still obscure.

In theory, remodelling can be fulfilled through two ways. One is to change the expression level of junction protein, but this requires a relatively long time (hours) to accomplish. Another mechanism is to dismantle proteins from the TJ complex through internalisation (endocytosis). There are different types of internalisation processes to remove proteins or to disassemble the intercellular junction complex (see review by Ivanov et al)[107]. The low-grade constitutive internalisation selectively dismantles certain proteins from TJs or AJs without compromising the integrity and function of a junction. Therefore, it is considered as a physiological internalisation. In some epithelia like intestinal cells treated with bacterial toxins, the TJ complex can be targeted to be disassembled without involvement of AJs. Sometimes both TJs and AJs can be internalised together, which induces a loss of cell attachment[107]. The pathways for the internalisation of TJ proteins include clathrin-mediated and caveolin-mediated endocytosis, as well as macropinocytosis[64,107]. However, which TJ protein is targeted and how the selection is made remain enigmatic. It is tempting to speculate the criteria for selecting the target proteins. It would not be surprising if a protein molecule is damaged or mutated, or if the cell needs to downregulate its membrane protein density quickly based on its function and metabolism.

The TJ proteins internalised are transported to early endosomes, most likely the apical early endosomes (AEEs) since the clathrin-coated pits, caveolae, or macropinosomes are formed and transported from the apical side. AEE delivers the proteins to the common recycling endosome (CRE) and later to be sorted for either re-inserting back to TJs or for degradation[107] (Fig 2). The mechanism and regulation of the sorting process in CREs remain poorly understood in epithelia in general and in particular, salivary epithelia.

Expression of TJ proteins in salivary epithelia

The expression of TJ proteins, especially claudins, is tissue and cell specific. It has been clearly demonstrated that different epithelia have differential expression of TJ proteins. One epithelium usually has more than two types of claudins co-expressed. Each claudin has unique barrier characteristics, including specific charge selectivity and size discrimination[108]. Thus, the overall property of TJs including distinct paracellular permeability to water, ions, and other molecules in a certain epithelium depends on the type and quantity of TJ proteins expressed in these cells[109-111]. Although the expression of TJ proteins in salivary glands has not been widely studied, a few reports provided an overall sketch of the spectrum of TJ proteins in several species (Table 2).

It is noteworthy that HSG cell line does not express claudin-1, -2, ZO-1, and occludin. As a result, these cells cannot effectively restrict paracellular leakage[117]. However, HSG cells grown on Matrigel express claudin-1, -2, -3, -4, JAM-A, occludin, and ZO-1, and form a sealed monolayer with a measureable TER[79].

The spectrum of TJ protein expression is altered when salivary cells are cultured *in vitro*. Qi et al[118] observed that the homogenate of rat parotid acinar cells contained claudin-3, -7 and occludin, although the level of claudin-7 was rather low. Claudin-4 and -6 were not detectable in the homogenate. Interestingly, the level of claudin-4 and -6 became significant after two to three days of primary culture. In turn, the pattern of mRNA expression was quite different. The mRNA levels of claudin-3 and -10 were significantly decreased during primary culture for three days, whereas claudin-4 and -6 mRNA were dramatically increased during the three-day culture.

In summary, human salivary glands express TJ membrane proteins claudin-1, -2, -3, -4, -5, -7, -11, JAM-A, and occludin. Surprisingly, rat PGs express only claudin-3, -4 and occludin. Mouse SMGs express claudin-3, -4, -5, -7, -8, -10, and -11. Obviously, this is not a complete list of TJ membrane proteins expressed in salivary glands since the purposes of these investigations were to measure only selected members of TJ membrane proteins. Further studies to reveal the whole spectrum of TJ protein expression in salivary glands are needed. Although this type of investigation seems to be descriptive, it will provide a solid basis for further exploration of the function and mechanisms of the TJ complex.

Paracellular fluid and ion transport

It is considerably complex to experimentally distinguish transcellular and paracellular pathways in a particular

Table 2 Expression of TJ proteins in salivary glands and cell lines

TJ protein	Expression site			
	Whole gland	Acini	Duct	Blood vessel
Claudin-1	Human SMG[26] Rabbit SMG[26]	Human PG and SMG[77] Human minor SGs[112]	Human PG and SMG[77] Human minor SGs[112] Human fetal SGs (4-24 weeks)[112] Rat SMG[113] HSG cells grown on Matrigel[78,79]	Human PG and SMG[77] Rat PG, SMG and SLG[114]
Claudin-2	Human SMG[26] Rabbit SMG[26]	Human PG and SMG[77]	Human PG and SMG[77] HSG cells grown on Matrigel[78,79]	
Claudin-3	Human SMG[26] Rabbit SMG[26]	Human PG and SMG[77] Human minor SGs[112] Rat PG, SMG and SLG[114] Mouse SMG (newborn)[115] SMG-C6 cells [71]	Human PG and SMG[77] Human minor SGs[112] Rat PG, SMG and SLG[114] Mouse SMG (newborn)[115] HSG cells grown on Matrigel[78,79] SMIE cells[113]	
Claudin-4	Human SMG[26]	Human PG and SMG[77] Human minor SGs[112] SMG-C6 cells[71]	Human PG and SMG[77] Human minor SGs[112] Human fetal SGs (4-24 weeks)[112] Rat PG, SMG and SLG[114] Mouse SMG (newborn)[115]	
Claudin-5	Human SMG[26] Rabbit SMG[26]	Human minor SGs[112] Human fetal SGs (4-24 weeks)[112] Rat PG, SMG and SLG[114] Mouse SMG (newborn)[115]	Human minor SGs[112] Human fetal SGs (4-24 weeks)[112] Mouse SMG (newborn)[115]	Rat PG, SMG and SLG[114]
Claudin-7	Human SMG[26] Rabbit SMG[26]	Human minor SGs[112] Mouse SMG (newborn)[115]	Human minor SGs[112] Human fetal SGs (4-24 weeks)[112] Mouse SMG (newborn) [115]	
Claudin-8		Mouse SMG (newborn)[115]	Mouse SMG (newborn)[115]	
Claudin-10		Mouse SMG (newborn)[115]		
Claudin-11	Human SMG[26] Rabbit SMG[26]	Human minor SGs[112] Mouse SMG (newborn)[115]	Human minor SGs[112] Human fetal SGs (4-24 weeks)[112]	
Claudin-16		Human SMG at the basal poles[116]	Human SMG[116]	
Occludin		Human PG and SMG[77] SMG-C6 cells[71]	Human PG and SMG[77] HSG cells grown on Matrigel[78,79] SMIE cells[113]	Human SMG[77]
JAM-A		Human PG[77]	Human SMG[77]	
ZO-1	Human SMG[26] Rabbit SMG[26]	Human PG and SMG[77]	Human PG and SMG[77]	Human PG and SMG[77]

PG: the parotid gland; SMG: the submandibular gland; SLG: the sublingual gland; SG: the salivary gland.

epithelium, such as salivary acini due to the lack of reliable methods to measure the amount or proportion of water movement through either pathway and universal existence of water and ions in all compartments, including all the intracellular and intercellular spaces. Since the 1970s, a large number of studies have focused on clarifying whether water transport was mediated by transcellular or paracellular pathway, but results remain inconclusive.

On the other hand, the characterisation of foreign chemical diffusion, such as drugs, across the epithelia seems to be more productive, as cells do not normally transport these agents *in vivo*. It is well known that the paracellular pathway is critical for drug absorption in the intestinal epithelia. Many studies used Caco-2 cell monolayers as a model measuring the contribution of paracellular and transcellular pathways for drug absorption. For example, transport of naproxen (molecular radius 3.69 Å), phenytoin (3.73 Å), salicylic acid (3.03 Å), propranolol (3.84 Å), diltiazem (4.62 Å), and ephedrine (3.43 Å) by Caco-2 cells at pH 7.2 was predominately through the transcellular pathway (97% to 99.9%), whereas cimetidine (3.81 Å), furosemide (3.91 Å), and chlorothiazide (3.61 Å) were substantially absorbed via the paracellular pathway (14.8%, 55.5%, and 74.3%, respectively)[119]. Similarly, ranitidine diffusion in Caco-2 cell monolayers is through both pathways in a concentration-dependent manner. At concentration of 0.1 mM, 40% transport is via transcellular pathway and 60% through paracellular pathway; at 0.5 mM, transcellular and paracellular portions become 55% and 45%, respectively; and at 2 mM, these proportions become 45% and 55%, respectively[120]. In general, hydrophobic compounds are transported mainly by the transcellular pathway due to their ability to easily traverse the cell membrane, whereas small hydrophilic solutes are diffused through the paracellular pathway[121,122]. Furthermore, the paracellular pathway has a much higher permeability to positively charged cations compared with neutral molecules or negatively charged anions[119].

To date, paracellular fluid and ion transport across TJs has been determined mainly by the measurement of tracer flux. The most frequently used tracers or probes include polar non-electrolytes, Lucifer yellow, dextrans and polyethylene glycols (PEGs). Analysing tracer flux data obtained by measuring the permeation of a series of tracers or probes with different molecular sizes can reveal characteristics of the barrier and fence function of the TJ complex, including junction permeability and pore radius. Many investigators in the areas of salivary glands and other epithelia have observed that two types of pores exist in TJs, small pores and large pores. The small pores are for ions to pass, given that these pores are size- and charge-selective channels[123-126], whereas the large pores are probably for larger nonionic molecules since they have no charge discrimination[123,124,127]. It remains uncertain which pathway mediates water flux.

Small pores

These pores are known under numerous terminology, including charge- and size-selective pores (or channels); charge- and size-restrictive pores; high-capacity and size-restrictive pores; simply small pores; restrictive pores; or high-capacity pores. Case et al[128] also referred to them as "fluid channels" since the pores were suggested to mediate both water and solute flux. However, our understanding of the structure, operation, and control of these pores is very limited after three decades of research.

Protein-function relationship of claudins

It is generally recognised that the small pores are formed by TJ membrane integral proteins, mainly by claudins. Increasing data indicate that the type and proportion of claudins decide the characteristics of pores. A large number of studies have provided some clue to the protein-function relationship of claudins. The following section summarises this relationship of claudins expressed in salivary glands.

The role of claudin-1 in paracellular fluid and ion transport remains inconclusive. Overexpression of claudin-1 in MDCK T23 cells increased TER, but the permeability to mannitol and 4-kDa FITC-dextran (FD) was also increased[89]. Knockout of claudin-1 in MDCK II cells did not affect TER, but Na^+ permeability was increased and Cl^- permeability decreased[129].

Claudin-2 has been frequently studied due to its role in mediating Na^+ flux. Expression of claudin-2 in MDCK II and MDCK C7 cells reduced TER and increased Na^+ permeability[34,36,130,131], and potentiated 3.5-Å PEG permeation[126]. Consistent with these results, knockdown of claudin-2 in MDCK II cells induced an increase in TER, a decrease in Na^+ permeability, and loss of cation selectivity[129].

The relationship between expression level of claudin-3 and fluid/ion transport is inconsistent in different epithelia. Expression of claudin-3 in MDCK II cells increased TER and reduced permeability to Na^+, Cl^-, fluorescein, and 4-kDa FD132, but the expression in rat alveolar cells decreased TER and enhanced calcein (0.6 kDa) and Texas Red dextran (10 kDa) permea-

tion[133]. Claudin-3 is expressed in salivary epithelia and may play a critical role in mediating fluid flux. AQP5$^{-/-}$ mice have a significantly lower claudin-3 expression and water transport[134]. Transplanted rabbit SMGs also have a dramatically reduced fluid secretion and claudin-3 expression compared to non-transplanted glands[26]. These results suggest claudin-3 may be one of the main proteins mediating the paracellular fluid transport.

Expression of claudin-4 in MDCK II, opossum kidney cells, rat alveolar cells, and SMG duct cell line SMIE increased TER[37,113,131,133] and Na^+ permeability in MDCK II and opossum kidney cells[37,131]. Knockdown of claudin-4 in MDCK II cells decreased TER and increased Na^+ permeation, and enhanced preference for cations[129]. Similarly, knockdown in LLC-PK1 cells increased TER without altering Na^+ permeability, but induced the loss of anion selectivity[129]. SMIE cells do not express claudin-4, therefore, gene transfer of claudin-4 in these cells increased TER and reduced 70-kDa dextran flux[113], indicating that this claudin is essential for tighter barrier function in salivary ducts.

The function of claudin-7 in paracellular ion transport is inconsistent in different cell lines. Experimental expression in LLC-PK1 cells increased Na^+ permeability[135] and knockdown reduced Na^+ permeation[129]. However, knockdown in MDCK II cells increased Na^+ permeability and enhanced preference for cations[129]. AQP$^{-/-}$ mice showed reduced claudin-7 expression and water flow in perfused salivary glands[134], indicating that claudin-7 may be critical in saliva secretion.

Despite claudin-11 expression in salivary glands, its function in paracellular fluid and ion transport is unknown. Cong et al[26] observed that the responses of claudin-11, along with claudin-1 and -3, to TRPV1 stimulation and to gland transplantation suggest it may be an important component of the TJ barrier in salivary epithelia.

Although the small pores are probably regulated by claudins, the relationship between claudin type and function of the pores remains poorly elucidated in salivary epithelia. Since salivary glands have both leaky and tight segments, they are the ideal system to explore the role of TJ proteins in paracellular fluid and ion transport.

Measurement of small pores

There are several classes of tracers or probes being widely used to characterise the small pores, including measuring apparent permeability and pore radius. These probes include small non-electrolytes, Lucifer yellow, dextrans, PEGs, and peptides. Other tracers or probes such as inorganic metals or nitrogenous cations have also been utilised, but their role in investigating small pores permeability is not extensive.

Small non-electrolytes

Many studies determined the flux of non-electrolytes with various molecular weights and hydrodynamic radii that may be suitable to reflect the paracellular water and ion movement. A basic requisite for non-electrolytes to be used as tracers is their incapability of moving through the transcellular pathway, i.e. not to traverse across the cell membrane to enter the cytoplasm. Various studies used isotope (^{14}C or ^{3}H)-labelled small non-electrolytes as tracers that do not cross cell membranes. Frequently used polar non-electrolytes include urea (hydrodynamic radius 2.71 Å), alanine (2.94 Å), glycerol (2.96 Å), creatinine (3.13 Å), erythritol (3.2 Å), mannitol (3.63 Å), L-dopa (3.72 Å), α-ME-dopa (3.81 Å), lactulose (4.56 Å), sucrose (4.56 Å), and raffinose (5.37 Å); positive charged tracer atenolol (4.15 Å); and negatively charge compounds formate (2.61 Å), acetate (2.71 Å), lactate (2.94 Å), foscarnet (3.22 Å), and hippurate (3.6 Å)[136]. All these tracers pass across the epithelia primarily through the paracellular pathway (> 90% of transepithelial flux is via paracellular transport). Some small non-electrolyte molecules have relatively high oil/water distribution coefficients, such as ethanol and antipyrine (both have an oil/water partition coefficient 32.0×10^{-3}) as well as thiourea (1.20×10^{-3}), which facilitates their entry into cells, thus quantifying the portion of transcellular versus paracellular transport is difficult.

Inorganic cations

A number of inorganic cations were used as tracers to determine the permeability of the paracellular fluid pathway, including Pb^{2+}, Ba^{2+}, Ag^+, and Tl^+. However, these tracers can gain entry into the cells and have toxic effects, greatly limiting their usage. Nonetheless, carefully designed experiments utilising these tracers provided valuable data about the structure and nature of the paracellular fluid transport pathway. Simson et al[18,137] used lead (Pb^{2+}) as a tracer to perfuse rat PGs through the blood circulation system for shorter than 2 minutes and directly observed lead precipitates in the acinar lumen via electron microscopy. With this method, differential permeability of TJs within a single secretory unit was measured. Lead precipitates were distributed primarily

in the extracellular space and did not pass through TJs between striated duct cells. The precipitates also were rarely found on the luminal side of TJs in the intercalated duct segment, but were easily observed in the lumen of acinar cells, indicating that TJs between acinar cells are permeable to the tracer, whereas those between striated duct cells are impermeable.

Lucifer yellow

Lucifer yellow ($C_{13}H_{10}Li_2N_4O_9S_2$, MW 444) is a cell impermeable chemical. It has been frequently used as a tracer for fluid secretion through the paracellular pathway[138,139]. Lucifer yellow is also often used to determine communication between cells since it can pass through the gap junctions.

Dextran

Another series of widely used probe for paracellular pathways is dextran. Dextran has different molecular weights with a strongly hydrophilic molecular structure that is theoretically difficult to diffuse across cell membranes to enter the cytoplasm. FITC-dextrans (or FDs) with various molecular weights are frequently used for characterisation of paracellular pathways, such as FD4 (MW 3.86 kDa), FD10 (9 kDa), FD20 (19.8 kDa), FD40 (40.5 kDa); FD70 (71.6 kDa), and FD150 (156.9 kDa). Many studies also utilised only one or two FDs as probes to explore the function and regulation of the paracellular fluid transport mechanism in salivary gland epithelia[20,26,96,97,134,140].

However, some studies observed that dextrans are able to enter cells and the permeability measured by using FDs may not accurately reflect paracellular transport. It is interesting to note that FDs have been used as markers of fluid-phase endocytosis. For example, Tomita et al[141] reported that FD4 passed through Caco-2 cell monolayers by a process that was inhibited by colchicine, an endocytosis inhibitor, and was reduced by unlabelled dextran (10 kDa), indicating that FD4 transport is mediated at least partially by endocytosis. Similar results were observed by Ohkubo et al[142] in Caco-2 cell monolayers. Although these experiments incubated cells with FD4 for an extended period (24 hours), the results demonstrated that FD4 can be internalised by Caco-2 cells. Bradbury and Bridges[143] utilised FD40 to monitor endocytosis in T84 cells by incubating cells with FD40 for only 5 minutes, suggesting that the dextran is able to enter cells in a short time period.

There also was evidence that certain conditions, such as Ca^{2+} depletion, enabled T84 cell monolayers to uptake rhodamine-conjugated dextran (10 kDa) via endocytosis[144]. Similar results were also reported in MDCK cells[145]. Incubation of MDCK cells with FD10 (10 kDa) for 15 or 30 minutes induced a clear FD uptake. Matsukawa et al[146] used a series of FDs with different MWs (from 3.86 kDa to 156.9 kDa) to measure paracellular transport in the alveolar epithelial monolayers and found that FDs with MWs smaller than 40 kDa pass through TJ pores with a radius of 50 Å, whereas larger molecules are transported across epithelial monolayers through pinocytosis. It remains unclear whether the uptake of FDs by salivary gland epithelial cells is similar to intestinal, kidney, and alveolar cells.

PEGs

PEGs are another class of widely used probe for measurement of permeability and radius of the small paracellular pathway. PEGs are neutral and water soluble. There are no reports indicating that these oligomers have any toxicity to cells. Most importantly, PEGs usually cannot traverse across cell membranes to enter the cytoplasm. It is well documented that transepithelial transport of PEGs is predominately via the paracellular pathway[147,148]. In general, the molecular size of PEG is proportional to its hydrodynamic radius. For example, PEG194 (the number in a PEG name indicates its MW), PEG282, PEG370, and PEG502 have a radius of 3.71Å, 4.24 Å, 4.71 Å, and 5.32 Å, respectively. To determine pore permeability and radius in an epithelium, it is necessary to use continuous series of PEGs. The amount of PEGs in samples can be separated by liquid chromatography and quantified by mass spectrometry. Recently, Van Itallie et al[126,149] developed a much simpler method that can separate and quantify modified PEGs by using HPLC and fluorescence emission.

The accuracy of using PEGs as probes for paracellular fluid or macromolecule transport has been challenged[150,151]. A typical example is that PEG194 was found to have a much (6~28 fold) higher permeability than mannitol, a widely used non-electrolyte tracer with a similar MW (182)[127]. The difference indicated that the more flexible PEG structures[127] have smaller radii than non-electrolytes or dextrans[125].

Pore size, permeability, and porosity of the small pores

One of the most important characteristics of paracellular pathways is the size or radius of the pores, which is the key factor allowing passage of molecules through the pores. Epithelial pore size is determined by using a series of tracers with different molecular size or hydrodynamic radius[126,152,153]. Using the results of tracer permeation across an epithelium or a cell monolayer, apparent permeability (P_{app}) and pore size (radius) can be determined by various equations[126,152,153,154].

The typical relationship between P_{app} and pore size or between fraction of volume flow or filtration coefficient and pore size is a hyperbola-like curve with higher values of P_{app} at smaller tracer radii, falling rapidly with the line flattening as the tracer radii increases (Fig 3)[126,128,154,155]. The curve clearly shows two existing components. The first segment of the curve with the steep negative slope is consistent with the small pores and the second segment of the curve with the flat slope is a reflection of the large pores.

Emerging data indicate that radii of small pores in various epithelia are similar, approximately 4~6 Å (Table 3). However, the number of pores in a unit area, i.e. the porosity or density of pores, varies significantly among epithelia, but is proportional to P_{app} of small tracers[126]. Van Itallie et al[126] used P_{app} values of 3.5 Å PEGs to reflect the density of pores in different epithelia or cell monolayers. Although pore size is consistent, pore density is tightly regulated by certain factors[126].

Case et al[128] measured the transport of 10 polar non-electrolytes in perfused rabbit mandibular glands and found that small polar non-electrolytes are transported across the epithelium through a type of pores with a radius of 4 Å. Howorth et al[155] perfused rabbit mandibular glands to study the permeability of small non-electrolytes, showing that the effective channel radius of the pores was about 4~4.5 Å. These results are similar to the reported radii of the small pores in rabbit gallbladder epithelia (3 Å)[156], kidney cell monolayers (4 Å), and intestinal cell monolayers (3.7~9 Å) (Table 3). It is postulated that a number of cations, such as Na^+, Ca^{2+} and probably Mg^{2+} may be transported through these paracellular pores in salivary acini, since the apical membrane does not appear to have a monovalent or divalent cation transport mechanism in accordance to the current transcellular fluid secretion model (Fig 1a).

A number of studies have determined the characteristics of the small pores. In general, monovalent cations (Li^+, Na^+, K^+, Rb^+, and Cs^+) have a much higher permeability than divalent cations (Ca^{2+} and Mg^{2+}) and the permeability is correlated with ion weight or size. Tang et al[163] observed that Na^+ permeability is constant when the Na^+ concentration gradient is in a range of 10~145 mM and divalent cation permeabilities are higher at low concentration gradients in MDCK II and T84 cells. Interestingly, when Na^+, Cl^-, Ca^{2+}, and Mg^{2+} are present simultaneously, equal ion permeability are observed in these cells[163]. When several cations coexist, the permeability to one cation is reduced, suggesting competition for the same pores[163].

The porosity or density of the small pores in the TJ complex remains unclear, despite its vital role in paracellular transport. Using a dual-pathway ultrastructural model for TJs in rat proximal tubule epithelium, Guo et al[164] found the density of small pores is one pore for every 20.2-nm TJ strands, suggesting that these pores play a critical role in NaCl transport since 91% of paracellular NaCl permeability was mediated by the small pores in rat proximal tubules[164]. Based on reported data that each TJ particle spreads approximately 20 nm along a TJ strand[165], it appears one TJ particle has one small pore in rat proximal tubule epithelium[164]. The apparent permeability of PEGs with molecular radius of 3.5 Å has been used to indirectly reflect density of small pores[126]. To date, there have not been any data indicating porosity of small pores in salivary gland epithelia.

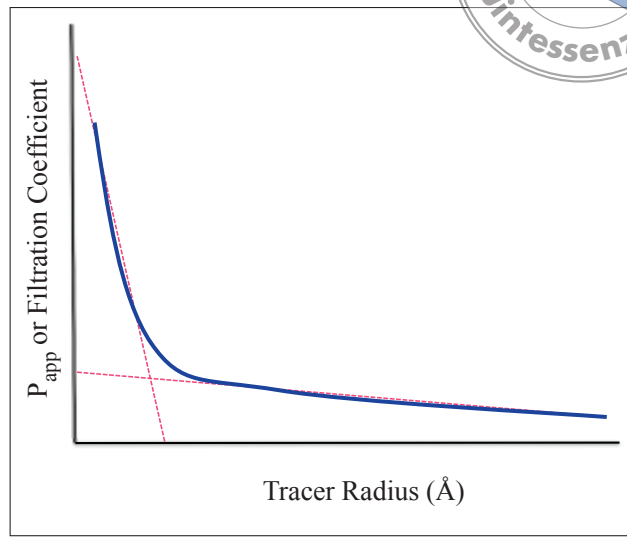

Fig 3 Schematic illustration of the relationship between apparent permeability (P_{app}) or filtration coefficient and molecular radii of tracers used in determination of paracellular transport. P_{app} or *filtration coefficient* is a function of tracer radii and rapidly decreases with tracer molecular radius increasing in cultured cell monolayers or in perfused salivary glands. (This figure is based on the models described by Van Itallie et al[126] and Shachar-Hill and Hill[154]).

Subtype of small pores

It is unclear whether small pores consist of different subtypes of pores or whether the size-selectivity and the charge-selectivity represent separate pores or channels. It is reasonable to hypothesise that size- and charge-selective pores are different subtypes and controlled by distinct operating mechanisms and regulating factors. Studies expressing a mutant TJ membrane protein dissociated paracellular permeability and TER[59,94,166],

Table 3 Radii of the small paracellular pores in salivary, intestinal, and kidney epithelia

Epithelium	Tracer	Pore Radius (Å)	Investigator, Year
Salivary gland			
Rabbit SM G	Non-electrolytes	4	Case et al, 1985[128]
Rabbit SMG	Non-electrolytes	4 ~ 4.5	Howorth et al, 1987[155]
Rat SMG	Non-electrolytes	3.8	Nakahari et al, 1996[157]
Rat SMG	Dextrans	5	Murakami et al, 2001[124]
Intestine			
Caco-2	Non-electrolytes	4.6	Knipp et al, 1997[123]
Caco-2	Peptides	5.1	Knipp et al, 1997[158]
Caco-2	PEGs	4.5	Watson et al, 2001[125]
Caco-2	Non-electrolytes	3.7	Tavelin et al, 2003[159]
Caco-2	PEGs	4	Van Itallie et al, 2008[126]
Caco-2	Non-electrolytes	< 4	Seki et al, 2008[160]
Caco-2	PEGs	5.8	Linnankoski et al, 2010[153]
Caco-2	Non-electrolytes	5.9	Kataoka et al, 2011[161]
T84	PEGs	4.3	Watson et al, 2001[125]
T84	PEGs	4.5	Watson et al, 2005[162]
3/4/A1	Non-electrolytes	9	Tavelin et al, 2003[159]
Pig ileum cells	PEGs	4	Van Itallie et al, 2008[126]
Human intestine	PEGs	6.6	Linnankoski et al, 2010[153]
Kidney			
MDCK II	PEGs	4	Van Itallie et al, 2008[126]
MDCK II	PEGs	5.5	Linnankoski et al, 2010[153]
MDCK C7	PEGs	4	Van Itallie et al, 2008[126]

SMG: the submandibular gland; MDCK: Madin-Darby canine kidney cell line.

indicating that size-selective pores and charge-selective channels are probably not similar.

Large pores

Although it has been well recognised that some macromolecules can pass the paracellular barrier to enter the lumen in exocrine gland epithelia, including salivary gland acini, the anatomical location of the large pores in the intercellular space remains controversial. Early studies proposed three possibilities to explain the observation of 'leakage' of macromolecules through the TJ complex: a) damage produced experimentally in epithelia or cell monolayers; b) a slit created by damaged, dying, or dead cells; and c) leaky and nonselective channels at the TJ complex[167]. The former two possibilities can be avoided by carefully performed experiments. It has been demonstrated that a temporary increase in permeability to

macromolecules can be induced by pathological stimuli such as proinflammatory cytokines in intestinal epithelia. This leakage pathway is formed by internalisation of TJ proteins[64]. The difference between this leakage and the large pores under physiological conditions needs to be examined. Accumulating data reveal that TJs contain a specific pathway for passage of large molecules such as peptides, proteins, lipids, and large probes (dextrans and PEGs). Guo et al[164] called the pathway 'large slit breaks'. Absorption of large hydrophilic peptides and proteins, such as drugs or antigens, in intestinal epithelia is mediated predominately by paracellular large pores. The large pores develop or mature earlier than smaller pores. It was observed that the large pores are present in Caco-2 cell monolayers cultured for 9 days, whereas the small pores appear in the cells cultured at 13 days[160].

Measurement of large pores

While small pores are measured with various small non-electrolyte tracers, the large pores have been determined with large molecular probes. The widely used molecular probes for measuring the large pores in 1970s and 1980s include microperoxidase (MW 1.68~1.9 kDa), cytochrome C (12 kDa), myoglobin (17.8 kDa), tyrosinase (34.5 kDa), horseradish peroxidase (HRP; 40 kDa), haemoglobin (66 kDa), and lactoperoxidase (82 kDa). For example, Garrett et al[168] injected retrograde HRP into the duct of SMGs in rabbits and observed that HRP was present in the interstitial space, indicating that the tracer traversed the TJ barrier. Most of these probes, typically HRP and lactoperoxidase, were less often used for measurement of paracellular transport due to their potential to induce cell membrane damage[169] and to enter cells if the incubation time is sufficient (1~4 hours)[170]. However, microperoxidase has been continuously used in measurement of TJ permeability in salivary glands as no adverse effect was observed[28,171]. One of the most frequently used probes for measurement of paracellular pathway to date is large FITC-dextran with various molecular weights, such as 4, 20, 40, or 70 kDa[26,113,134,140].

Permeability of large pores

In general, the apparent epithelial permeability to large molecular probes is adversely proportional to the molecular size of the probes. Early studies found that rat parotid glands are not permeable to HRP at rest conditions, but TJs between acinar cells are permeable to HRP administered via the intraductal lumen at physiological pressures (<15 mm Hg) following secretagogue stimulation, whereas the junctions between striated duct cells are more resistant to passage of the probe[172]. Kuijpers et al[173] found that the paracellular barrier of rabbit pancreas acini is permeable to HRP. Using FITC-dextrans with MW of 3.86, 9, 19.8, 40.5, 71.6 and 156.9 kDa to measure paracellular transport in alveolar epithelial monolayers, Matsukawa et al[174] observed that the apparent permeability of low-MW dextran is higher compared to high-MW dextran. Overall, it is generally assumed that the permeability of large pores is much lower compared to small pores.

Size and density of large pores

To date, there are no consistent data about the size of large pores. Watson et al[125] suggested that the radius may be far larger than 7.4 Å based on the size of the largest PEG oligomer used in their measurements. Seki et al[160] suggested the pore radius to be much larger than 21 Å in Caco-2 cells. Matsukawa et al[174] found that the radius of large pores in alveolar cell monolayers is 50 Å; macromolecules smaller than 40 kDa can pass through the pores, whereas molecules larger than 40 kDa are transported cross the epithelium via pinocytosis. Linnankoski et al[153] reported that radius of the large pores is 30.5 Å in MDCK II cells, 10.4 Å in Caco-2 cells, 14.9 Å in 2/4/A1 cells, and 10.1 Å in human intestine epithelia. Guo et al[164] found that the large slit breaks are 172 nm in length and 19.6 nm (196 Å) in height in rat proximal tubules. Similar large slit breaks with a length of 150 nm and 200 nm have also been observed in frog mesentery capillary1[75] and rat urinary tubules[165], respectively. Using protein probes, microperoxidase, cytochrome C, myoglobin, tyrosinase, hemoglobin, HRP, and lactoperoxidase to measure the permeability of rat parotid gland TJs, Mazariegos et al[169] found that any molecules large than 1.9 kDa cannot pass through the paracellular pathway of the rat parotid gland at rest conditions. This molecular weight has become a reference for the size limit of paracellular transport and has been cited frequently.

The density of the large pores is quite low, 8×10^{-8} in MDCK II cells, 7×10^{-8} in Caco-2 cells, 1.54×10^{-6} in 2/4/A1 cells, and 8.9×10^{-7} in human intestine epithelia[153]. Guo et al[164] found that the frequency of the large slit breaks is 1.94% in rat proximal tubules. However, there has not been any specific investigation characterising similar large pores in salivary gland epithelia.

In summary, although various studies have demonstrated the existence of small pores and large pores as the paracellular pathway in TJs, the structure of these pores are unclear. For example, how claudins form

charge- and size-restrictive pores remains unrevealed. In theory, studies focusing on amino acid residues of claudin proteins may provide valuable clues to the issue. Through testing and manipulating amino acid sequences of a specific claudin, the role of the protein sequence may be delineated, though these experiments could be difficult. In addition, more questions arise related to the large pores; for example, how the pores are formed; whether claudins are involved; how the pores can selectively open for macromolecule passage without permitting water, ion or small non-electrolyte diffusion; and how these pores are regulated under physiological and pathological conditions. Several models have been proposed to depict the paracellular ion transport through the small pores[106,154], but there is no model proposed for macromolecule transport. Further studies to clarify these issues will have a high impact on the mechanisms of the paracellular pathway.

Water transport

Transepithelial water transport is the key issue for elucidation of the mechanism of fluid transport. However, after decades of investigation and debate, the issue remains highly controversial.

Transcellular or paracellular?

The debate on which pathway mediates water passage across an epithelium, i.e. the transcellular or the paracellular pathway, has lasted for decades. Elucidation was expected when aquaporin, the so-called 'water channel' proteins, were identified and sequenced. However, the finding of aquaporin did not provide a clear resolution to this issue. AQP5 is the major aquaporin expressed in the apical membrane of salivary acinar cells and is proposed as the primary transcellular water pathway[176-178]; however, elimination of AQP5 in knockout mice ($AQP5^{-/-}$) did not completely eliminate water secretion from salivary glands. In $AQP5^{-/-}$ mice, saliva secretion was reduced by 60%[179] and water permeability was reduced by 65% in parotid acinar cells and 77% in isolated sublingual acinar cells[180]. Incomplete deletion of water secretion in $AQP^{-/-}$ mice suggests existence of other water transport pathway(s) that is not related to aquaporin.

In other epithelia such as the intestine, water was observed to be cotransported by antiporters (exchangers), uniporters, and cotransporters, such as the K^+-Cl^- or the Na^+-K^+-$2Cl^-$ cotransporters and the glucose uniporters, often referred as 'the cotransporter model'[181-183]. This mechanism of water transport is energised by coupling to the substrate flux and is able to transport water uphill against an osmotic gradient[181-183]. Salivary acini have several types of ion exchangers and the Na^+-K^+-$2Cl^-$ cotransporter; therefore, it would not be surprising if water is transported into the cell through these ion transporters. However, water secretion across the apical membrane into the lumen is unlikely to be mediated by such mechanism since no ion exchanger or cotransporter has been identified in the apical membrane of salivary acini.

Studies by Murakami et al[124,184], using perfused rat SMGs, provided evidence that the paracellular pathway was the primary pathway for water secretion and the transcellular pathway mediated only a small fraction of water flux. Segawa et al[139] used confocal microscopy to visualise Lucifer yellow fluorescence to reflect water movement in arterially perfused rat PG and SMG, observing that the initial water secretion after muscarinic stimulation was through the transcellular pathway, and the sustained water flux was via the paracellular pathway. Nakahari and Imai[157] used a digitised imaging technique to monitor the changes in acinar cell volume and the swelling of the lumen space in perfused rat SMG. Stimulation of the glands induced transient swelling of the luminal space before the acinar cell shrinkage, indicating that fluid secreted into the lumen was not from a cellular source, instead it was from the paracellular pathway. However, Kawedia et al[134] studied paracellular and transcellular water transport in $AQP^{-/-}$ and wild type ($AQP^{+/+}$) mice by using 4-kDa dextran D as a tracer. After injecting dextran D via the jugular vein to $AQP^{-/-}$ and $AQP^{+/+}$ mice, whole saliva was collected following stimulation with parasympathetic agonist pilocarpine. The results of the experiments indicate that transcellular pathway was the major fluid transport pathway in mouse salivary glands.

Hill and Shachar-Hill proposed that AQP5 is not the channel mediating water flow, but an osmotic sensor and a regulator of the paracellular water pathway[154,185,186]. The osmosensor model hypothesises that water flow rate is regulated by aquaporins localised in the apical (and basolateral) membrane, detecting the osmotic and turgor pressure gradient and providing feedback to the junctional complex, adjusting water permeability. A study conducted by Kawedia et al[134] found that deletion of AQP5 reduced both TJ protein expression and paracellular water flux, providing partial support for Hill's osmosensor hypothesis. Currently, the questions whether fluid transport is mainly mediated by transcellular or paracellular pathway and what role aquaporin plays in fluid transport remain unaddressed.

Need osmotic or ionic gradient?

Most fluid transport models propose that paracellular water flux needs an osmotic gradient or an electro-osmotic gradient as the driving force to attract water and ion movement[187-190]. However, the osmosensor feedback model proposed by Hill and Shachar-Hill1[54,185,186] hypothesised that epithelia, including the salivary gland secretory epithelium, transport most solutes through transcellular pathway, with water and some ions movement through a hypotonic paracellular pathway, not requiring an osmotic or electro-osmotic gradient. A number of articles using mathematical models to demonstrate these theories have been published and the debate on whether an osmotic gradient is required for paracellular fluid transport is at an impasse. Nonetheless, most experimental data suggest that an osmotic gradient may be needed for paracellular fluid movement. Murakami et al[191] used arterially perfused rat SMG to explore the effect of hydrostatic pressure on the paracellular transport of Lucifer yellow and found that at least part of the paracellular fluid transport is driven by hydrostatic pressure. Nakahari et al[192] perfused rat SMG with perfusates at different osmolarities, and found that fluid movement across the salivary epithelia was dependent on the osmotic difference between the lumen and the interstitium. A recent study by Rosenthal et al[130] investigated the role of osmotic and Na^+ gradients in fluid transport in claudin-2-expressed MDCK cells and found that water flow across the paracellular barrier is driven by two factors, osmotic and Na^+ gradients, with an additive effect observed when both gradients are simultaneously applied to the system.

The pathway mediating water flow

Since the molecular radius of water is much smaller than the radius of small pores and solvent drag may be the mechanism mediating water movement, it is reasonable to assume that high-capacity small pores should be the pathway for water flux, whereas large pores may play a minor role in mediating water passage[128,155,184]. Howorth et al[155] found that there is approximately 2% water flow through the large pores in salivary epithelia. Rosenthal et al[130] observed that claudin-2 forms paracellular water channels in MDCK C7 cells. However, contrasting data have also been observed. Guo et al[164] found that the infrequent large slit breaks (large pores) mediate 95% of paracellular water permeability in rat proximal tubules.

Fluid transport mechanism

Early studies proposed that the major mechanism that mediates non-electrolytes movement from the interstitium to the lumen of salivary gland acini is by solvent drag, which couples solute transport and water flow by a fractional interaction between solute and water[128]. In other words, water flux drags solute movement. This mechanism becomes evident in many studies. For example, Murakami et al[184] found that Lucifer yellow movement across the paracellular pathway was proportional to fluid secretion in perfused rat SMGs, indicating that its transport is by solvent drag. Rosenthal et al[130] also provided evidence that solvent drag is the mechanism by which water and ions are transported since expression of claudin-2 increased both Na^+ and water transport, whereas claudin-10b was not able to mediate water flux, resulting in no increase in Na^+ transport when expressed. It also has been shown that the primary mechanism of Ca^{2+} absorption in the intestine is mediated by solvent drag[193-195]. For example, 70% of duodenal Ca^{2+} absorption is through this mechanism[193].

Regulation of the paracellular pathway

In contrast to transcellular secretion, there have not been systemic investigations on the regulation of the paracellular fluid, electrolyte and macromolecule transport. However, a number of studies have explored the possible controlling mechanisms of the paracellular pathway. The following section will provide a brief summary of current studies conducted on the possible mechanisms and factors that regulate TJ structure and function.

General regulatory mechanisms

The TJ structure and function are regulated by many systems, including the autonomic nervous system, the endocrine, paracrine, and autocrine systems. Furthermore, many other factors such as nutrients, therapeutic chemicals, and environmental contaminants also impact TJ function. These factors may act through common or different mechanisms.

Phosphorylation of TJ proteins

Phosphorylation of some TJ proteins is a common, and possibly the primary way to rapidly regulate TJ structure and function. Most TJ signalling proteins are able to phosphorylate other TJ proteins, specifically the transmembrane proteins, i.e. claudins, TAM proteins, and JAMs.

Phosphorylation of claudins

Claudins can be acutely phosphorylated, rapidly altering TJ barrier function. PKA, PKC, MAPK, and Rho kinase can phosphorylate various claudins, resulting in a change in TER and permeability to water and ions (see review by Angelow et al[14]). The latter depends mainly on which claudin is involved. For example, PKA-induced phosphorylation of claudin-3 at Thr192 in ovarian cells caused a decrease in TER[196]. Phosphorylation of claudin-5 at Thr207 by PKA in rat lung endothelial cells rapidly reduced TER and increased mannitol flow[197]. The TJ pores specific for Mg^{2+} are formed by claudin-16 and the pore permeability is regulated by phosphorylation of claudin-16 at Ser217; any factor that induces dephosphorylation of claudin-16, such as PKA inhibitors, adenylate cyclase inhibitors, or point mutant at Ser217, can abolish PKA effects[198].

Phosphorylation of occludin

Occludin is not only an integral component of TJs, but also a regulator of TJ structure and function. Occludin exists in multiple phosphorylation states in the cell and the majority of the highly phosphorylated occludin is concentrated in the TJ complex[199]. It is hypothesised that occludin plays a critical role as a signalling or regulatory platform altering TJ formation, function, and disassembly and merging various signalling systems regulating TJs, such as under oxidative and reducing conditions[200]. For example, phosphorylation of occludin on Tyr residues in C-terminal domain reduces its interaction with ZO-1; in contrast, phosphorylation of Ser or Thr residues may increase the interaction, leading to regulation of TJ assembly or disassembly[201]. Phosphorylation of occludin by protein kinase CK2 alters TJ structure and permeability[202]. When occludin is phosphorylated at Ser408 by CK2, occludin-claudin-2 complex formation is blocked and claudin-2 is able to constitute charge- and size-selective pores, permitting Na^+ passage. If occludin is not phosphorylated by CK2, it forms a complex with ZO-1 and claudin-2, thus claudin-2 loses its ability to form Na^+ pores[203] (see review by Shen[202]). Therefore, phosphorylation of occludin is a critical step for TJ regulation.

Phosphorylation of JAMs

Phosphorylation of JAMs by PKC at Ser284 or 285 is needed for the development of TJ barrier function[204,205]. However, the effects of JAM phosphorylation in TJ structure and function have not been extensively studied.

Phosphorylation of cytoskeletal proteins

Cytoskeletal proteins are not TJ integral membrane proteins, but TJs are associated with the cytoskeleton. In the 1980s, ultrastructural studies already demonstrated that TJs are associated with actin filaments. Disruption of actin filaments by cytochalasin D or phalloidin induces structural changes in TJs (see review by Rodgers and Fanning[206]). However, TJ integral membrane proteins do not directly bind to cytoskeletal proteins; their association is mediated by scaffolding proteins ZO-1, -2, -3, or cingulin, though cingulin may play only a minor role. It has been repeatedly demonstrated that disruption of the cytoskeleton alters TER and paracellular permeability. Part of this effect may be induced by the contraction of the perijunctional actomyosin ring, which theoretically enlarges the intercellular space between adjacent cells. A number of studies indicated that myosin II appears to be responsible for this effect. It is well established that myosin light chain (MLC) is involved in the regulation of paracellular permeability. This is likely through MLC phosphorylation[206]. More recently, it has been clearly demonstrated that MLC phosphorylation is a critical step in the regulation of TER and paracellular transport in the SMG-C6 cell line[71].

Internalisation of TJ proteins

Another rapid regulation of TJ structure and function is the internalisation of TJ proteins. As mentioned above, the TJ complex is a highly dynamic structure and exists in a state of continuous remodeling. Claudins seem to be relatively stable in TJs. For example, it has been reported that 60% of claudin-1 molecules are immobile in the TJ complex in intestinal epithelia compared to 30% of occludin proteins. Most ZO-1 molecules are in the state of continuous exchanging between the TJ complex and the cytosol[207] (see review by Shen[202]). The underlying mechanism regulating TJ protein remodeling remains unclear. Cong et al[71] observed that stimulation of the TRPV1 receptors induced a redistribution of occludin between the TJ complex and the cytosol, a decrease in TER, and an increase in permeability to trypan blue and 4-kDa FITC-dextran in 5 minutes, the shortest time point examined in SMG-C6 cells. These changes depend on ERK1/2 activation.

Changing TJ protein expression

The long-term regulation of TJ structure and function is the alteration of TJ protein expression. This has been demonstrated frequently in many studies, although the regulating mechanism may be different in various epithelia. For example, exposure of Par-C10 cells to TNF-α and IFN-γ induced a decrease in the expression of claudin-1[20]. It is observed that ZO-1 and occludin

are strongly downregulated, while claudin-1 and -4 are overexpressed in labial salivary glands from patients with Sjögren syndrome[30]. Stimulation of TRPV1 receptors with capsaicin induced a significant increase in the expression of ZO-1, claudin-3 and -11 in rabbit SMG cells, which was coupled with increased TER and decreased paracellular permeability to tracers[26].

In general, changing protein expression is a slow and long-term regulation, which takes hours to accomplish. Activation of the TRPV1 receptor increased expression of ZO-1, claudin-3 and -11 mRNAs in 12 hours and increased the expression of proteins in 24 hours[26]. These were the shortest time points determined and the accurate time needed following expression of these mRNAs and proteins may be shorter, requiring further studies. In contrast, upregulation of members of the signal transduction system mediating these responses was faster. Capsaicin-stimulated expression of ERK1/2 and MLC2 took place following 5 minutes[26], indicating they also play a part regulating the rapid responses.

Physiological regulation

Neurotransmitters

Saliva section is controlled by the autonomic nervous system, mainly the sympathetic and parasympathetic nerves. Specifically, under physiological conditions, water and ion transport is elicited predominately by muscarinic agonist acetylcholine and α_1-adrenergic agonist norepinephrine, whereas the secretion of proteins and other organic macromolecules is evoked mainly by β-adrenergic agonist isoproterenol[6-9]. Although the non-adrenergic, non-cholinergic (NANC) peptide agonists, such as vasoactive intestinal peptide (VIP), substance P (SP), and neuropeptide Y (NPY), also participate in the regulation of salivary secretory responses[208], their roles in saliva secretion are relatively minor. The nucleotide transmitters, such as ATP and UTP, are able to trigger strong secretory responses, including Ca^{2+} mobilisation and ion transport in isolated or cultured salivary cells[209-211], but their action *in vivo* is relatively minimal under physiological conditions. Although controlling or regulation of the transcellular fluid, electrolyte, and protein secretion is extensively investigated, our knowledge about the paracellular pathway for water, electrolyte, and macromolecule transport remains essentially limited.

Cholinergic and α_1 adrenergic agonists

It is postulated that the paracellular pathway is under the same regulation as the transcellular pathway since the salivary secretion is a well-synchronised process. A large number of studies stimulated perfused salivary glands or cultured cell monolayers by either stimulation of the chorda tympani nerve or administration of muscarinic agonists to elicit fluid and ion flux through the paracellular pathway (Table 4). It is well accepted that stimulation of muscarinic cholinergic system triggers an increase in paracellular fluid and ion diffusion, however, the signalling system mediating the response remains undefined.

As it is well known that Ca^{2+} is the main signal for transcellular ion transport, it would not be unreasonable to assume that Ca^{2+} should play a substantial role in controlling paracellular water and ion flow. However, a number of studies have focused on the effects of extracellular Ca^{2+} on TJ regulation[219,220]. Neither an increase in cytosolic Ca^{2+} concentration with Ca^{2+} ionophore A23187 or with specific endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin or a reduction in cytosolic Ca^{2+} with specific chelator BAPTA alters TJ opening or closing states in frog urinary bladders[219], indicating that TJs may not be directly regulated by intracellular Ca^{2+}. Very few studies have focused on this key issue in salivary epithelia. On the other hand, extracellular Ca^{2+} change has a profound influence on TJ function. Depletion of extracellular Ca^{2+} causes TJ opening or loosening, loss of TER, and TJ protein internalisation. Restoration of Ca^{2+} results in the opposite changes in TJ function[219].

PKC is activated when stimulated with muscarinic agonists and modulates Ca^{2+} signalling system in rat SMG cells[221]. It has been demonstrated that PKC extensively participates in the regulation of TJ formation, function, and dynamic remodeling[222,223], including phosphorylation of ZO-1, occludin, and claudins[222]. Furthermore, TJ opening and closing may be tightly regulated by PKC[223]. Since the stimulation of muscarinic receptors induces hydrolysis of the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) by the enzyme phospholipase C (PLC) to produce diacylglycerol (DAG) and inositol trisphosphate (IP_3) to function as second messengers, the activation of PKC is parallel to activation of Ca^{2+} movement in salivary cells. It is possible that PKC may be a key factor in the signalling system that regulates TJs. Further investigation to test this hypothesis would be highly beneficial.

The role of α_1-adrenergic agonists in the regulation of TJ formation, structure, function, or remodelling is unknown. Mazariegos and Hand[170] stimulated rat PG with α_1-adrenergic agonist methoxamine and did not induce myoglobin (17.8 kDa) passage through TJs. Muscarinic agonist methacholine and combination of

Table 4 Regulation of Paracellular Permeability by the Autonomic Nervous System or Neurotransmitters in Perfused Salivary Glands or Cultured Salivary Cells

Species and Gland	Stimulation	Response
Rat SMG[171]	Electric stimulation of the chorda tympani nerve (20 Hz)	Microperoxidase (1.63 kDa) entering the lumen
Rat SMG[212]	Electric stimulation of the chorda tympani nerve and/or the superior cervical ganglion	Microperoxidase and HRP entering the lumen
Dog SMG[213]	Electric stimulation of the chorda lingual nerve	HRP entering saliva
Rabbit SMG[128]	Acetylcholine	Increase in paracellular diffusion of water and small polar non-electrolytes
Rabbit SMG[155]	Acetylcholine	Increase in paracellular permeability to water and non-electrolytes
Rabbit SMG[214]	Acetylcholine	Increased in fluid secretion, but no change in transcellular water flow
Rat PG[170]	Methacholine and/or methoxamine	Impermeable to tracer myoglobin (17.8 kDa)
Rat SMG[157]	Acetylcholine	Increase in water flow via the paracellular pathway
Rat PG and SMG[215]	Carbachol	Tracers ≤10 kDa pass through the paracellular pathway
Rat PG and SMG[139]	Carbachol	Water flows through the paracellular pathway after 30-45 seconds of stimulation
Rat SMG[124]	Acetylcholine	Increase in paracellular diffusion of water and ions
Rabbit SMG[26]	Capsaicin (TRPV1 agonist)	Increase in 4-kDa FITC-dextran entering the lumen; upregulated expression of claudin-3 and -11, and ZO-1; redistribution of F-actin
SMG-C6 cells[71]	Capsaicin (TRPV1 agonist)	Increase in trypan blue and 4-kDa FITC-dextran diffusion across TJs; decrease in TER of cell monolayers; occludin internalization
Rat PG[170]	Isoproterenol (1-4 hours)	Increase in myoglobin passing through TJs
Rat PG[169]	Isoproterenol (1-4 hours)	TJs are impermeable to molecules >1.9 kDa; stimulation with isoproterenol increases TJ permeability to molecules up to 34 kDa.
Rat PG and SMG[215]	Isoproterenol	Increase in tracers with MW ≤40 kDa passing through the paracellular pathway
Rat SMG[138]	Carbachol + Isoproterenol	Lucifer yellow entering the lumen
Rat SMG[216]	Electric stimulation of chorda tympani nerve (20 Hz)	HRP entering saliva, which is blocked by substance P antagonist
Rat SMG[217]	Substance P	Microperoxidase entering the lumen
Rat SMG[218]	Neurokinin A	Increase in paracellular Lucifer yellow diffusion

PG: the parotid gland; SMG: the submandibular gland; HRP: horseradish peroxidase

both methoxamine and methacholine did not enhance the permeability to myoglobin (Table 4), indicating that muscarinic and α_1-adrenergic stimulation does not activate the large pores.

β Adrenergic agonists

Several studies using perfused salivary glands demonstrated that stimulation of the sympathetic nervous system or β-adrenergic receptors induced macromolecules that were impermeable when unstimulated to pass through the TJs (Table 4). Obviously, the β-adrenergic agonist opens the large pores. Since the characteristics of the large pores are not well studied, little is known about the regulatory mechanisms. Stimulation of β-adrenergic receptors stimulates cAMP production, which in turn activates PKA. It has been reported that PKA induces the phosphorylation of claudin-3 at Thr192 and regulates the TJ barrier function in ovarian cells[196]. PKA also phosphorylates claudin-5 at Thr207, which induces a decrease in TER and an increase in mannitol diffusion in rat lung endothelial cells[197]. PKA also can directly phosphorylate claudin-16 and increase Mg^{2+} reabsorption in MDCK cells[198]. All these effects regulate the small pores. While this varies from results observed in salivary glands, it provides solid evidence for PKA regulation of TJ functions. Further exploration of the relationship between PKA activation and regulation of large pores in salivary epithelia is of significant importance.

NANC peptides

The significance of NANC receptors in saliva secretion under physiological conditions is unclear. There were a few earlier studies indicating that NANC receptors have the potential to regulate paracellular fluid and ion movement. So far, only substance P has been studied.

Takai et al[216] observed that electric stimulation of the chorda tympani nerve induced infused HRP entry into saliva in rat SMGs; not inhibitable by muscarinic antagonist atropine, but reduced significantly by substance P antagonist (Table 4). In substance P-depleted glands, electric stimulation of parasympathetic nerves failed to evoke HRP entry. Therefore, it was concluded that the increased TJ permeability to HRP was mediated by substance P[216]. Tani et al also observed that substance P induced microperoxidase entry into the lumen in rat SMGs[217]. However, the effects observed in these studies are on the large pores and the second messenger for substance P receptors is Ca^{2+}. Further studies to address this inconsistency and to examine the roles of other NANC receptors in regulating paracellular transport are needed.

TRPV1 agonists

Activation of TRPV1 receptors with capsaicin stimulates 4-kDa FITC-dextran entry into the lumen of acini, increase in expression of claudin-3, -11, and ZO-1, and redistribution of F-actin in rabbit SMG[26] (Table 4). Similarly, stimulation of SMG-C6 cell monolayers with capsaicin induced a decrease in TER, an increase in trypan blue and 4-kDa FITC-dextran diffusion across TJs, and redistribution of occludin from the TJ complex to the cytosol. These effects are mediated by ERK1/2-induced phosphorylation of MLC2[71]. These results suggest that MAPK/ERK1/2 may be the major signalling system regulating the paracellular pathway in salivary epithelia. Further studies to address the question how ERK1/2 is activated by capsaicin stimulation, i.e., the upper stream elements of the signal cascade, will be needed.

Regulation by hormones

The influence of hormones on saliva secretion has not been extensively or systematically studied to date. The actions of hormones on paracellular transport in salivary epithelia remain basically unknown. We will briefly discuss only a few hormones that are likely to be associated with saliva secretion and paracellular transport.

Glucocorticoids

Glucocorticoids promote differentiation of proacinar cells and stimulate production of secretory granules in the developing rat SMG[224]. However, there have not been studies testing the effects of glucocorticoids on the paracellular transport function in salivary epithelia, although it has been examined in other epithelia.

Incubation of human bronchial epithelial cell lines 16HBE and Clau-3 with 0.01~1 μM synthetic glucocorticoids dexamethasone (DEX), fluticasone propionate, or budesonide for 5 days significantly increased monolayer TER and decreased permeability to 4-kDa or 10-kDa FITC-dextran. Immunofluorescent microscopy demonstrated that TJ formation was enhanced in DEX-treated cell monolayers, although expression of occludin and ZO-1 was not altered[225]. Similar effects was also observed in the mouse mammary cell HC11, where 1 μM DEX induced an significant and continuous increase in TER and a decrease in ^{3}H-inulin diffusion and occludin expression was also increased following 5 days of incubation[226]. Glucocorticoids also influence intestinal epithelia. A critical pathogenic factor for intestinal inflammation, such as Crohn disease, is TJ barrier dysfunction. Glucocorticoids are often used as therapeutic agents to counter inflammation and can restore intestinal TJ function, such as preventing

TNF-α-induced permeability increase in Caco-2 cell monolayers[227]. DEX also can inhibit hypoxia-induced TJ dysfunction, such as decreased ZO-1 expression in human corneal epithelium[228].

The mechanism by which glucocorticoids enhance or protect epithelial TJ function has been explored in various cell types. The effects of DEX in human bronchial cells appear to be mediated by increased phosphorylation of epithelial growth factor (EGF) receptors and the knockdown of EGF receptors significantly reduced DEX effects[225]. Glucocorticoids' effects also may be through potentiated TJ formation, since DEX effects were observed in MDCK cells only during the TJ barrier formation[229]. On the other hand, the protection of TJ function in Coca-2 cells by DEX was through binding an activated glucocorticoid receptor complex stimulating myosin light chain kinase (MLCK) expression[227].

It is well known that glucocorticoids modulate epithelial Na^+ channels (ENaC) and transepithelial Na^+ transport. Salivary cell line SMG-C6 monolayers grown in a medium lacking glucocorticoids had significantly lower amiloride-sensitive short-circuit current (I_{sc}) and addition of 1.1 μM hydrocortisone restored I_{sc}[230]. However, it remains unclear whether glucocorticoids play a similar role in paracellular Na^+ or other ion diffusion in salivary epithelia.

Mineralocorticoids

The regulatory roles of mineralocorticoids, primarily aldosterone, on reabsorption of Na^+ and water as well as secretion of K^+ and H^+ in the kidney have been well characterised, but the role of aldosterone in the regulation of paracellular fluid and ion diffusion is unclear. Aldosterone has been demonstrated to regulate saliva secretion, as it induces an increase in K^+ and decrease in Na^+ content, thus altering the salivary Na^+/K^+ ratio secreted by red kangaroo mandibular glands[231] and bovine parotid glands[232]. However, it remains unclear whether aldosterone executes these actions through altering paracellular pathway in salivary glands.

In the intestine and the kidney, aldosterone has a clear impact on paracellular ion transport. Incubation of rabbit distal colon epithelia, both the low-transporting and high-transporting epithelia, with 0.1 μM aldosterone on the serosal side nearly doubled I_{sc}. The paracellular resistance was also significantly increased by aldosterone in both epithelia[233]. In adrenalectomised rat distal colonic mucosa, aldosterone induced an increase in TER and Na^+ accumulation and a decrease in dextran permeability, whereas infusion of angiotensin II did not alter the effects of aldosterone, suggesting that aldosterone enhances Na^+ absorption and reduces paracellular permeability[234]. The increased Na^+ absorption by aldosterone is probably mediated by the transcellular pathway. A measurement of the paracellular resistance with two-path impedance spectroscopy in intestinal cell HT-29/B6-GR monolayers indicated that aldosterone-induced increase in paracellular barrier function is secondary to the upregulation of claudin-8 expression, preventing Na^+ back leakage down the Na^+ gradient in the epithelial cells. Simultaneously, $^{22}Na^+$ transport by ENaC was increased in response to aldosterone[235].

The potential underlying mechanisms that mediate aldosterone effects has been studied in renal collecting duct cell RCCD2. Aldosterone induced a transient phosphorylation of claudin-4 on threonine residues without affecting claudin-4 expression or localisation in the TJ complex[236]. This effect appears aldosterone-specific since it required mineralocorticoid receptor occupation and DEX did not elicit a similar response. Aldosterone also increased ^{125}I passage, reflecting Cl^- diffusion, whereas $^{22}Na^+$ diffusion was unchanged[236].

In summary, aldosterone regulates transepithelial ion movement by increasing ENaC activity, increasing Na^+ transport by stimulating expression of tight claudins such as claudin-4 and -8 as well as increasing paracellular permeability in intestinal and renal epithelia. These results may shed light on the regulatory effects of aldosterone in paracellular fluid and ion transport in salivary epithelia.

Androgen

Salivary gland cells have receptors for sex steroids; however, their roles on salivary secretory function have not been well studied. One of the innovative hypotheses for the etiology and pathogenesis of Sjögren syndrome is an insufficient local androgen level in exocrine (mainly salivary) gland tissues[237]. The local androgen is produced by intracrine reactions, converting dehydroepiandrosterone (DHEA) into dihydrotestosterone (DHT) within the exocrine cells. A DHEA supplement to patients with Sjögren syndrome increased serum DHEA, free testosterone, androstenedione, and DHT, but local androgen depletion was not corrected due to the intracrine failure. This primarily affects females, who must produce DHT and testosterone locally in salivary glands[238]. It has been observed that DHT stimulates initial TJ formation and function in Sertoli cells in mice[239] and DHT-deficient rats exhibited intercellular junction disintegration, including a decrease in cadherin, β-catenin, and occludin, which underlies the formation of premature germ cells in the seminiferous epithelium[240]. It is unclear whether such a decrease in DHT production in salivary cells has any detrimental effects on TJs. This should be a focus of further exploration.

Vitamin D

Vitamin D (VD) is a hormone playing a major role in regulating Ca^{2+} homeostasis. The active form of VD, 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] is the most important factor controlling Ca^{2+} absorption in the intestine. However, it is unclear if $1,25(OH)_2D_3$ has similar effect on Ca^{2+} transport in the salivary acini and ducts. It has been evident for years that salivary epithelia of PG, SMG, and SLG in various species are the major targets of VD[241] and that saliva secretion is significantly decreased in VD-deprived rats[242,243]. Ca^{2+} absorption stimulated by $1,25(OH)_2D_3$ in intestinal epithelia is mainly (70%) through the paracellular pathway[193]. However, contradictory results were found whether VD was capable of potentiating the saturable (transcellular) or nonsaturable (paracellular) Ca^{2+} transport *in vivo*. Recently, Fujita et al[244] demonstrated, by using VD receptor (VDR)-knockout mice, that $1,25(OH)_2D_3$ stimulates claudin-2 and -12 expression in Caco-2 cells and a lack of VDR significantly reduced claudin-2 and -12 expression and Ca^{2+} transport in rat intestines. $1,25(OH)_2D_3$ binds to its nuclear receptors and activates genomic processes, increasing the transcription of proteins stimulating transcellular Ca^{2+} transport, such as the plasma membrane Ca^{2+}-ATPase, TRPV6, and the Ca^{2+}-binding protein calbindin[245]. However, this is not a rapid process and requires hours to complete. The effects of $1,25(OH)_2D_3$ on the paracellular Ca^{2+} diffusion occur in a much shorter time period. Incubation of isolated rat intestine segments with $1,25(OH)_2D_3$ induced a rapid concentration-dependent increase in paracellular Ca^{2+} flux, whereas transcellular Ca^{2+} transport was not changed[195,246]. This enhancement of Ca^{2+} diffusion was abolished by the inhibitor of phosphatidylinositol-3-kinase (PI3K), PKC, and MEK, suggesting that the stimulation by $1,25(OH)_2D_3$ is mediated by these protein kinases[195]. Saliva contains a considerable level of Ca^{2+} (about 41 mg/L), but its source and secretory mechanism remain unclear. Investigations to address these important issues are needed. Hypothetically, the pathway and mechanisms by which Ca^{2+} is secreted by salivary endpieces could be explored by using cultured cell monolayers and perfused glands in special claudin-knockout or knockdown cell lines and animal models.

Growth factors

Growth factors have been reported to play a critical part in maintaining the TJ structure and paracellular transport function.

Epidermal growth factor

Various studies found that epidermal growth factor (EGF) regulates TJ function by altering claudin expression and degradation. Singh and Harris[247] observed that EGF inhibited claudin-2 expression and increased expression of claudin-1, -3, and -4, but had no influence on ZO-1, occludin and AJ proteins E-cadherin and β-catenin in MDCK cells. These changes led to a 3-fold increase in TER[247]. Similar results were observed by Ikari et al[248,249], who further elucidated the intracellular signalling system mediating these changes. Inhibition of endocytosis with monodancylcadaverine or by reducing clathrin with clathrin siRNA, or inhibition of lysosomal protease with chloroquine blocked EGF-induced claudin-2 decrease, indicating that the underlying mechanism is an increase in claudin-2 degradation. These effects are mediated by activation of ERK1/2[248,249].

EGF can also protect TJs from acetaldehyde-induced damage, including a decrease in TER, an increase in inulin permeation, and a redistribution of junction proteins occludin, ZO-1, E-cadherin, and β-catenin in Caco-2 cells. Furthermore, the protective effects of EGF were mediated by the ERK1/2 signalling system[250]. Similar protective effects were observed in bile duct epithelium, inhibiting hydrogen peroxide (H_2O_2)-induced disruption of TJs and AJs and loss of barrier function. Pretreatment with EGF significantly reduced these damages. However, the intracellular signalling system mediating the protective effects by EGF in these cells was a phosphoinositide phospholipase C (PLC)-γ and PKC-dependent mechanism[251].

The ERK1/2 signalling system has been demonstrated to mediate TRPV1 agonist-stimulated protection from transplantation (denervation)-induced hypofunction in human and rabbit SMGs[26,71]. It is highly likely that the ERK1/2 signalling system may be the common signalling pathway for regulating TJ structure and paracellular transport. Further development in this area will be conducive to a full understanding of the regulatory network.

Insulin-like growth factor-I

Studies have confirmed that insulin-like growth factor-I (IGF-I) expressed in mouse[252], rat[253], and human[254] salivary glands. Mitsui et al[140] cultured SMIE cells in media containing either 10% foetal bovine serum (FBS) or different concentrations of IGF-I and found that IGF-I stimulates cell growth and maintains the paracellular barrier function. TER of SMIE cell monolayers was significantly reduced and the permeability to 4-kDa dextran significantly increased when cells cultured in a medium without FBS or IGF-I; whereas both TER and

dextran permeability exhibited an IGF-I concentration-dependent recovery when cells were cultured in medium containing IGF-I.

EGF and IGF-I are autocrine or paracrine products that can act on the cells of origin or near the signal-releasing cell. It is well established that salivary glands synthesise these growth factors; however, it is unclear if they execute their actions on salivary epithelia. Further exploration of the role of these growth factors in salivary gland function will be highly significant.

Pathological regulation

Cytokines

Cytokines are one of the major etiological factors in autoimmune diseases such as Crohn disease, cystic fibrosis, and Sjögren syndrome. Proinflammatory cytokines alter TJ structure and function in various epithelia. The salivary glands of Sjögren syndrome patients produce increased proinflammatory cytokines and decreased anti-inflammatory cytokines[255]. The effects of two major proinflammatory cytokines, TNF-α and IFN-γ, on TJ structure and function have been studied in many epithelia, including salivary epithelia. Chronic exposure of Par-C10 cell monolayers to TNF-α and IFN-γ induced a decrease in TER and the carbachol- or UTP-stimulated anion secretion, an increase in paracellular permeability to proteins, and downregulation of claudin-1[20]. TJ protein levels in labial salivary glands of Sjögren syndrome patients were significantly decreased compared to normal glands obtained from control subjects. ZO-1 and occludin were downregulated and claudin-1 and -4 overexpressed; furthermore, ZO-1 and occludin were decreased in the TJ area and claudin-3 and -4 were redistributed to the basolateral plasma membrane[30]. Importantly, treatment of normal acini with TNF-α and IFN-γ induced alterations similar to those observed in these patients[30].

Effects of other cytokines, such as interleukins (IL), on epithelial TJs in intestinal epithelia, but not in salivary epithelia, have been extensively studied[256,257]. For example, IL-7 expression in salivary glands of patients with Sjögren syndrome is upregulated, which induces increased inflammation[258], but its influence on TJ structure and paracellular transport has not been examined.

The intracellular signalling system mediating the effects of cytokines has not been studied in salivary cells. However, TNF-α-, IL-4-, and IFN-γ-induced dissociation of ZO-1 and occludin and TER change in airway epithelial cell line Calu-3 are mediated by the EGF receptor-dependent MAPK, specifically ERK1/2 pathway[259].

Oxidative stress

Oxidative stress is a common factor for many inflammatory diseases, including autoimmune disorders, such as Sjögren syndrome. Oxidative stress in inflammation originates from reactive oxygen species (ROS) released by immune cells, such as phagocytes. Oxidative reactions are increased in patients with Sjögren syndrome[260,261]. Oxidative stress disrupts TJs and increases paracellular permeability in a variety of epithelia. Using H_2O_2 to experimentally induce oxidative stress, some studies have found that activation of the MAPK/ERK system prevents oxidative stress-induced disruption of TJs in Caco-2 cells[262] and enhances recovery of TJ structure and function in MDCK II cells[263]. This effect is not surprising since it has been frequently reported that ERK1/2 is needed for regulation of paracellular permeability in MDCK cells[264,265]. The influence of oxidative stress on TJ structure and paracellular fluid transport in salivary cells remains unexamined.

In summary, TJ formation, remodelling, and function are regulated by neurotransmitters, hormones, as well as paracrine and autocrine peptides, under physiological conditions. Many pathological factors also have profound influence on TJs. The intracellular signalling systems mediating physiological stimuli and pathological insults are rather complex. It is highly likely that multiple signalling pathways coexist, which raises an arduous but intriguing challenge, i.e. to elucidate the roles of the signalling members and to synchronise them into a signalling network. Although it is premature to predict the network based on current knowledge on the issue, we would like to try to hypothesise the potential interaction of the signals reported (Fig 4).

Conclusion and future directions

The TJ is a dynamic structure sealing the intercellular space at the apical side, a barrier and selective channels for water and ion movement, and a fence and platform for regulating cell polarity; it is essential for normal function of epithelia and endothelia. The last two decades have been a fruitful period in TJ research with many critical findings in TJ formation, structure, remodelling, function, regulation, and roles in diseases. These new findings have greatly enhanced our view on the physiology of epithelia, endothelia, and immune cells. The continuous remodelling of TJ structure plays enormous parts in water and electrolyte transport in various epithelia, including salivary acini and ducts. The term "simple diffusion" used for a long period to describe paracellular transport in saliva secretion is no longer simple; in fact,

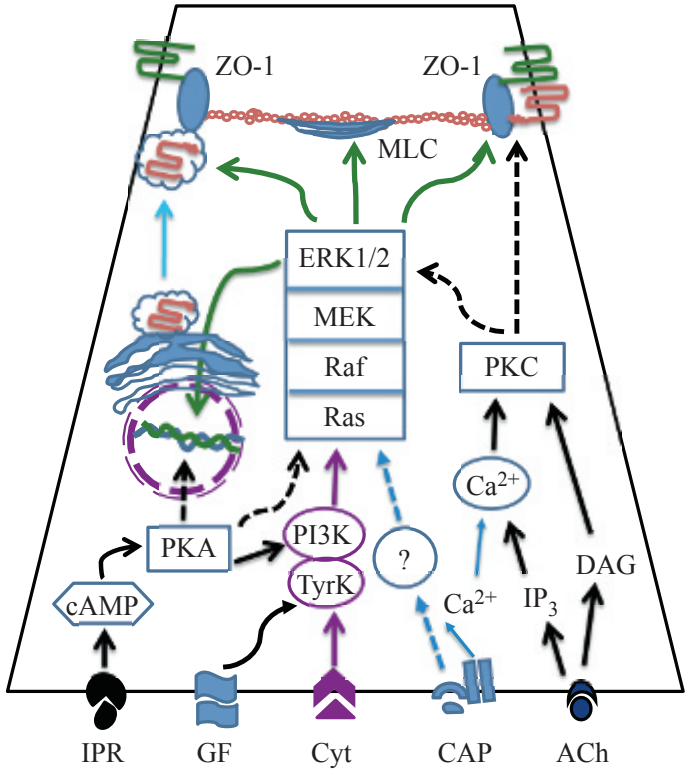

Fig 4 Potential signalling network regulating TJ remodelling and function in salivary epithelia. Stimulation of muscarinic receptors with acetylcholine (ACh) induces production of diacylglycerol (DAG) and inositol trisphosphate (IP_3). IP_3 triggers increase in cytosolic free Ca^{2+}, and DAG activates protein kinase C (PKC). Ca^{2+} also can activate the classical PKC. PKC may directly phosphorylate TJ proteins to alter their function. PKC also activates the Ras-Raf-MEK-ERK mitogen-activated protein kinase (MAPK) system, although the experimental evidence is needed. Activation of the transient receptor potential vanilloid subtype 1 (TRPV1) with capsaicin (CAP) also activates the MAPK system, especially ERK1/2. ERK1/2 plays a central role in regulating TJ remodelling and function. Through phosphorylation, ERK1/2 regulates 1) the association state of occludin and ZO-1, which is considered as a regulatory platform, adjusting the opening and closing state of the paracellular pores formed by claudins; 2) the internalisation and reinsertion of TJ membrane proteins, mainly occludin; 3) the contraction of actin myosin and rearrangement of the cytoskeleton structure; and 4) the expression of TJ proteins. Stimulation of β-adrenergic receptors with isoproterenol (IPR) induces the production of cAMP, which activates the cAMP-activated protein kinase (PKA). PKA may activate the expression of TJ proteins and/or the MAPK system. Growth factors (GF) and proinflammatory cytokines (Cyt) activate growth factor receptors and tyrosine kinase (TyrK). TyrK activates phosphatydylinositol-3-kinase (PI3K); the latter activates MAPK/ERK1/2. Other physiological or pathological stimuli, such as reactive oxygen species, may interact with any member of this network, leading to activation or inhibition of ERK1/2.

it is strikingly complex. However, our understanding regarding the characteristics of TJs in salivary epithelia remains elemental. It can be reasonably predicted that further research in TJs and paracellular transport mechanism will dramatically change the model of saliva secretion in the near future.

Future focus of investigations on TJs and paracellular transport could include studies exploring the TJ protein expression model, especially claudin expression; the relationship between claudin type and water/ion permeability, eventually leading to a settlement on the four-decade debate, finally deciding which pathway, transcellular or paracellular, mediates water flow; the significance, composition, structure, and regulation of the large pores mediating macromolecules; the roles and mechanisms of protein complexes, such as occludin-ZO-1 complex and cytoskeleton protein-ZO-1 complex; the regulatory process of TJ formation and remodelling; the signalling systems regulating TJs; the interaction between TJs and AJs; and the pathological factors that inhibit TJ function or disrupt TJ structure. These studies may pave a way to the development of novel and effective therapies for salivary gland dysfunction and other diseases.

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References

1. Thaysen JH, Thorn NA, Schwartz IL. Excretion of sodium, potassium, chloride and carbon dioxide in human parotid saliva. Am J Physiol 1954;178:155–159.
2. Martinez JR, Holzgreve H, Frick A. Micropuncture study of submaxillary glands of adult rats. Pflüg Arch 1966;290:124–133.
3. Mangos JA, Braun G. Excretion of total solute, sodium and potassium in the saliva of the rat parotid gland. Pflüg Arch 1966;290:184–192.
4. Young JA, Schögel E. Micropuncture investigation of sodium and potassium excretion in rat submaxillary saliva. Pflüg Arch 1966;291:85–98.
5. Hill AE. Fluid transport: A guide for the perplexed. J Membr Biol 2008;223:1–11.
6. Nauntofte B. Regulation of electrolyte and fluid secretion in salivary acinar cells. Am J Physiol Gastrointest Liver Physiol 1992;263: G823–G837
7. Martinez JR. Cellular mechanisms underlying the production of primary secretory fluid in salivary glands. Crit Rev Oral Biol Med 1993;1:67–78.
8. Cook DI, Van Lennep EW, Roberts ML, et al. Secretion by the major salivary glands. In: Johnson LR (ed): Physiology of the Gastrointestinal Tract. New York, Raven. 1994:1061–1117.
9. Melvin JE, Yule D, Shuttleworth T, et al. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Ann Rev Physiol 2005;67:44–69.
10. Silva P, Stoff J, Field M, et al. Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. Am J Physiol Renal Fluid Electrolyte Physiol 1977;233:F298–F306.
11. Cook DI, Young JA. Effect of K^+ channels in the apical plasma membrane on epithelial secretion based on secondary active Cl^- transport. J Membr Biol 1989;110:139–146.
12. Zhang GH, Cragoe EJ, Melvin JE. Regulation of cytosolic pH in rat subligual mucous acini at rest and during muscarinic stimulation. J Membr Biol 1992;129:311–321.
13. Nguyen HV, Shull GE, Melvin JE. Muscarinic receptor-induced acidification in sublingual mucous acinar cells: loss of pH recovery in Na^+-H^+ exchanger-1 deficient mice. J Physiol 2000;523: 139–146.
14. Angelow S, Ahlstrom R, Yu ASL. Biology of Claudins. Am J Physiol Renal Physiol 2008;295:F867–F876.
15. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. Cold Spring Harb Perspect Biol 2009;1:a002584.
16. Baker OJ. Tight junctions in salivary epithelium. J Biomed Biotech 2010;2010:278948.
17. Claude P. Morphological factors influencing transepithelial permeability: A model for the resistance of the zonula occludens. J Membr Biol 1978; 39:219–232.
18. Simson JAV, Bank HL. Freeze-fracture and lead ion tracer evidence for a paracellular fluid secretory pathway in rat parotid glands. Anet Rec 1984;208:69–80.
19. Kikuchi K, Kawedia J, Menon AG, et al. The structure of tight junctions in mouse submandibular gland. Anat Rec 2010;293:141–149.
20. Baker OJ, Camden JM, Redman RS, et al. Proinflammatory cytokines tumor necrosis factor-α and interferon-γ alter tight junction structure and function in the rat parotid gland Par-C10 cell line. Am J Physiol Cell Physiol 2008;295: C1191–C1201.
21. Schmidt KL, Henagan JM, Smith GS, et al. Effects of ethanol and prostaglandin on rat gastric mucosal tight junctions. J Surg Res 1987;43:253–263.
22. Bodi N, Talapka P, Poles MZ, et al. Gut region-specific diabetic damage to the capillary endothelium adjacent to the Myenteric Plexus. Microcirculation 2012;19:316–326.
23. Aberdeen GW, Wiegand SJ, Bonagura TW, et al. Vascular endothelial growth factor mediates the estrogen-induced breakdown of tight junctions between and increase in proliferation of microvessel endothelial cells in the baboon endometrium. Endocrinology 2008;149:6076–6083.
24. Leach L, Firth JA. Fine structure of the paracellular junctions of terminal villous caprillaries in the perfused human placenta. Cell Tissue Res 1992;268:447–452.
25. Nighot PK, Blikslager AT. CIC-2 regulates mucosal barrier function associated with structural changes to the villus and epithelial tight junction. Am J Physiol Gastrointest Liver Physiol 2010;299 :G449–G456.
26. Cong X, Zhang Y, Shi L, et al. Activation of transient receptor potential vanilloid subtype 1 increases expression and permeability of tight junction in normal and hyposecretory submandibular gland. Lab Invest 2012;92:753–768.
27. Capaldo CT, Macara IG. Depletion of E-cadherin disrupts establishment but not maintenance of cell junctions in Madin-Darby canine kidney epithelial cells. Mol Biol Cell 2007;18:189–200.
28. Murakami H, Uchihashi K, Yoshida Y. Regulated permeability of tight junctions by E-cadherin in rat submandibular gland acini. J Osaka Dent Univ 1999;33:23–33.
29. Cereijido M, Contreras RG, Flores-Benitez D, et al. New diseases derived or associated with the tight junction. Arch Med Res 2007;38:465–478.
30. Ewert P, Aguilera S, Alliende C, et al. Disruption of tight junction structure in salivary glands from Sjögren's syndrome patients is linked to proinflammatory cytokine exposure. Arth Rheum 2010;62: 1280–1289.
31. Furuse M, Fujita K, Hiiragi T, et al. Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 1998;141:1539–1550.
32. Mineta K, Yamamoto Y, Yamazaki Y, et al. Predicted expansion of the claudin multigene family. FEBS Lett 2011;585:606–612.
33. Furuse M. Knockout animals and natural mutations as experimental and diagnostic tool for studying tight junction functions in vivo. Biochim Biophy Acta 2009;1788:813–819.
34. Amasheh S, Meiri N, Gitter AH, et al. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. J Cell Sci 2002;115:4969–4976.
35. Van Itallie CM, Fanning AS, Anderson JM. Reversal of charge selectivity in cation or anion-selective epithelial lines by expression of different claudins. Am J Physiol Renal Physiol 2003;285: F1078–F1084.
36. Furuse M, Furuse K, Sasaki H, et al. Conversion of zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. J Cell Biol 2001;153: 263–272.
37. Van Itallie C, Rahner C, Anderson JM. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. J Clin Invest 2001;107:1319–1327.
38. Wen HJ, Watry DD, Marcondes MCG, et al. Selective decrease in paracellular conductance of tight junctions: role of the first extracellular domain of claudin-5. Mol Cell Biol 2004;24:8408–8417.
39. Yu As, Enck AH, Lencer WI, et al. Claudin-8 expression in Madin-Darby canine kidney cells augments the paracellular barrier to cation permeation. J Biol Chem 2003;278:17350–17359.
40. Furuse M, Hata M, Furuse K, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 2002;156:1099–1111.

41. Muto S, Hata M, Taniguchi J, et al. Claudin-2-deficient mice are defective in the leaky and cation-selective paracellular permeability properties of renal proximal tubules. Proc Natl Acad Sci USA 2010;107: 8011–8016.
42. Tamura A, Hayashi H, Imasato M, et al. Loss of claudin-15, but not claudin-2, causes Na^+ deficiency and glucose malabsorption in mouse small intestine. Gastroenterology 2011;140:913–923.
43. Delfín DA, Xu Y, Schill KE, et al. Sustaining cardiac claudin-5 levels prevents functional hallmarks of cardiomyopathy in a muscular dystrophy mouse model. Mol Ther 2012;20:1378–1383.
44. Anderson WJ, Zhou Q, Alcalde V, et al. Genetic targeting of the endoderm with claudin-6. Dev Dyn 2008;237:504–512.
45. Tatum R, Zhang Y, Salleng K, et al. Renal salt wasting and chronic dehydration in claudin-7-deficient mice. Am J Physiol Renal Physiol 2010;298:F24–34.
46. Ding L, Lu Z, Foreman O, et al. Inflammation and disruption of the mucosal architecture in claudin-7-deficient mice. Gastroenterology 2012;142:305–315.
47. Wilcox ER, Burton QL, Naz S, et al. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. Cell 2001;104:165–172.
48. Ben-Yosef T, Belyantseva IA, Saunders TL, et al. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. Hum Mol Genet 2003; 12:2049–61.
49. Nakano Y, Kim SH, Kim HM, et al. A claudin-9-based ion permeability barrier is essential for hearing. PLoS Genet 2009;5:e1000610.
50. Breiderhoff T, Himmerkus N, Stuiver M, et al. Deletion of claudin-10 (Cldn10) in the thick ascending limb impairs paracellular sodium permeability and leads to hypermagnesemia and nephrocalcinosis. Proc Natl Acad Sci USA 2012;109:14241–14246.
51. Gow A, Southwood CM, Li JS, et al. CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell 1999;99:649–659.
52. Gow A, Davies C, Southwood CM, et al. Deafness in Claudin 11-null mice reveals the critical contribution of basal cell tight junctions to stria vascularis function. J Neurosci 2004;24:7051–7062.
53. Tamura A, Kitano Y, Hata M, et al. Megaintestine in claudin-15-deficient mice. Gastroenterology 2008;134:523–534.
54. Will C, Breiderhoff T, Thumfart J, et al. Targeted deletion of murine Cldn16 identifies extra- and intrarenal compensatory mechanisms of Ca^{2+} and Mg^{2+} wasting. Am J Physiol Renal Physiol 2010;298: F1152–F1161.
55. Himmerkus N, Shan Q, Goerke B, et al. Salt and acid-base metabolism in claudin-16 knockdown mice: impact for the pathophysiology of FHHNC patients. Am J Physiol Renal Physiol 2008;295: F1641–F1647.
56. Shan Q, Himmerkus N, Hou J, et al. Insights into driving forces and paracellular permeability from claudin-16 knockdown mouse. Ann N Y Acad Sci 2009;1165:148–151.
57. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2001;2:285–293.
58. Furuse M, Fujimoto K, Sato N, et al. Overexpression of occludin, a tight junction-associated integral membrane protein, induces the formation of intracellular multilamellar bodies bearing tight junction-like structures. J Cell Sci 1996;109:429–435.
59. Balda MS, Whitney JA, Flores C, et al. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. J Cell Biol 1996;134:1031–1049.
60. McCarthy KM, Skare IB, Stankewich MC et al. Occludin is a functional component of the tight junction. J Cell Sci 1996;109:2287–2298.
61. Furuse M, Itoh M, et al. Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 1993;123:1777–1788.
62. Yu AS, McCarthy KM, Francis SA, et al. Knockdown of occludin expression leads to diverse phenotypic alterations in epithelial cells. Am J Physiol Cell Physiol. 2005;288: C1231–C1241.
63. Shen L, Turner JR. Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. Mol Biol Cell 2005;16: 3919–3936.
64. Utech M, Mennigen R, Bruewer M. Endocytosis and recycling of tight junction proteins in inflammation. J Biomed Biotech 2010;doi:10.1155/2010/484987
65. Hirase T, Staddon JM, Saitou M, et al. Occludin as a possible determinant of tight junction permeability in endothelial cells. J Cell Sci 1997;110:1603–1613.
66. Moroi S, Saitou M, Fujimoto K, et al. Occludin is concentrated at tight junctions of mouse/rat but not human/guinea pig Sertoli cells in testes. Am J Physiol Cell Physiol 1998;274:C1708–C1717.
67. Saitou M, Furuse M, Sasaki H, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol Biol Cell 2000;11:4131–4142.
68. Shultzke JD, Gitter AH, Mankertz J, et al. Epithelial transport and barrier function in occludin-deficient mice. Biochim Biophys Acta 2005;1669:34–42.
69. Ikenouchi J, Sasaki H, Tsukita S, et al. Loss of occludin affects tricellular localization of tricellulin. Mol Biol Cell 2008;19:4687–4693.
70. Krug SM, Amasheh S, Richter JF, et al. Tricellulin forms a barrier to macromolecules in tricellular tight junctions without affecting ion permeability. Mol Biol Cell 2009;20:3713–3724.
71. Cong X, Zhang Y, Yang N-Y, et al. Occludin is required for transient receptor potential vanilloid subtype 1-modulated paracellular permeability in submandibular gland. J Cell Sci 2013; http://www.ncbi.nlm.nih.gov/pubmed/23345400.
72. Ikenouchi J, Furuse M, Furuse K, et al. Tricellulin constitutes a novel barrier at tricellular ontacts of epithelial cells. J Cell Biol 2005;171:939–945.
73. Mariano C, Sasaki H, Brites D, et al. A look at tricellulin and its role in tight junction formation and maintenance. Eur J Cell Biol 2011;90:787–796.
74. Westphal JK, Dorfel MJ, Krug SM, et al. Tricellulin forms homomeric and heteromeric tight junctional complexes. Cell Mol Life Sci 2010;67:2057–2068.
75. Raleigh DR, Marchiando AM, Zhang Y, et al. Tight junction-associated MARVEL proteins marveld3, tricellulin, and occludin have distinct but overlapping functions. Mol Biol Cell 2010;21:1200–1213.
76. Steed E, Rodrigues NT, Balda MS, et al. Identification of MarvelD3 as a tight junction-associated transmembrane protein of the occludin family. BMC Cell Biol 2009;10:95.
77. Maria OM, Kim J-WM, Gerstenhaber JA, et al. Distribution of tight junction proteins in adult human salivary glands. J Histochem Cytochem 2008;56:1093–1098.
78. Maria OM, Zeitouni A, Gologan O, et al. Matrigel improves functional properties of primary human salivary gland cells. Tissue Eng Part A 2011;17:1229–1238.
79. Maria OM, Maria O, Liu Y, et al. Matrigel improves functional properties of human submandibular salivary gland cell line. Int J Biochem Cell Biol 2011;43:622–631.
80. Ebnet K, Aurrand-Lions M, Kuhn A, et al. The junctional adhesion molecule (JAM) family members JAM-2 and JAM-3 associate with the cell polarity protein PAR-3: a possible role for JAMs in endothelial cell polarity. J Cell Sci 2003;116:3879–3891.
81. Mandell KJ, Parkos CA. The JAM family of proteins. Adv Drug Deliv Rev 2005;57:857–867.
82. Laukoetter, MG, Nava P, Lee WY, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. J Exp Med 2007;204: 3067–3076.
83. Ebnet K, Suzuki A, Ohno S, et al. Junctional adhesion molecules (JAMs): more molecules with dual functions? J Cell Sci 2004;117:19–29.

84. Severson EA, Parkos C. Structural determinants of junctional adhesion molecule A (JAM-A) function and mechanisms of intracellular signaling. Curr Opin Cell Biol 2009;21:701–707.
85. Mirza M, Pang M-F, Zaini MA, et al. Essential role of the coxsackie- and adenovirus receptor (CAR) in development of the lymphatic system in mice. PLos One 2012;7:e37523.
86. Raschperger E, Thyberg J, Pettersson S, et al. The coxsackie- and adenovirus receptor (CAR) is an in vivo marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis. Exp Cell Res 2006;312:1566–1580
87. Su L, Mruk DD, Cheng CY. Regulation of the blood-testis barrier by coxsackievirus and adenovirus receptors. Am J Physiol Cell Physiol 2012;303:C843–C853.
88. Masuda S, Oda Y, Sasaki H, et al. LSR defines cell corners for tricellular tight junction formation in epithelial cells. J Cell Sci 2011;124:548–555.
89. McCarthy KM, Francis SA, McCormack JM, et al. Inducible expression of claudin-1-myc but not occludin-VSV-G results in aberrant tight junction strand formation in MDCK cells. J Cell Sci 2000;113:3387–3398.
90. Bauer H, Zweimueller-Mayer J, Steinbacher P, et al. The dual role of zonula occludens (ZO) proteins. J Biomed Biotech 2010;2010:402593.
91. Gonzalez-Mariscal L, Bautista P, Lechuga S, et al. ZO-2, a tight junction scaffold protein involved in the regulation of cell proliferation and apoptosis. Ann NY Acad Sci 2012;1257:133–141.
92. Xu J, Kausalya PJ, Phua DC, et al. Early embryonic lethality of mice lacking ZO-2, but not ZO-3, reveals critical and non-redundant roles for individual zonula occludens proteins in mammalian development. Mol Cell Biol 2008;28:1669–1678.
93. Hernandez S, Chavez MB, Gonzalez-Mariscal L. ZO-2 silencing in epithelial cells perturbs the gate and fence function of tight junctions and leads to an atypical monolayer architecture. Exp Cell Res 2007;313: 1533–1547.
94. Van Itallie CM, Fanning AS, Bridges A, et al. ZO-1 stabilizes the tight junction solute barrier through coupling to the perijunctional cytoskeleton. Mol Biol Cell 2009;20:3930–3940.
95. Kawedia JD, Jiang M, Kulkarni A, et al. The protein kinase A pathway contributes to Hg^{2+}-induced alterations in phosphorylation and subcellular distribution of occludin associated with increased tight junction permeability of salivary epithelial cell monolayers. J Pharmacol Exp Ther 2008;326:829–837.
96. Fujita-Yoshigaki J, Matsuki-Fukushima M, Sugiya H. Inhibition of Src and p38 MAP kinases suppresses the change of claudin expression induced on dedifferentiation of primary cultured parotid acinar cells. Am J Physiol Cell Physiol 2008;294:C774–C785.
97. Fujita-Yoshigaki J. Analysis of changes in the expression pattern of claudins using salivary acinar cells in primary culture. Methods Mol Biol 2011;762:245–258.
98. Li D, Mrsny RJ. Oncogenic Raf-1 disrupts epithelial tight junctions via downregulation of occludin. J Cell Biol 2000;148:791–800.
99. Hamm-Alvarez SF, Chang A, Wang Y, et al. Etk/Bmx activation modulates barrier function in epithelial cells. Am J Physiol Cell Physiol 2001;280:C1657–C1668.
100. Takai Y, Nakanishi H. Nectin and afadin: novel organizers of intercellular junctions. J Cell Sci 2003;116:17–27.
101. Ooshio T, Fujita N, Yamada A, et al. Cooperative roles of Par-3 and afadin in the formation of adherens and tight junctions. J Cell Sci 2007;120:2352–2365.
102. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nature Rev 2001;2:285–293.
103. Heiskala M, Peterson PA, Yang Y. The roles of claudin superfamily proteins in paracellular transport. Traffic 2001;2:93–98.
104. Muto S, Furuse M, Kusano E. Claudins and renal salt transport. Clin Exp Nephrol 2012;16:61–67.
105. Sasaki H, Matsui C, Furuse K, et al. Dynamic behavior of paired claudin strands within apposing plasma membranes. Proc Natl Acad Sci USA 2003;100:3971–3976.
106. Steed E, Balda MS, Matter K. Dynamics and functions of tight junctions. Trends Cell Biol 2010;20:142–149.
107. Ivanov AI, Nusrat A, Parkos CA. Endocytosis of the apical junctional complex: mechanisms and possible roles in regulation of epithelial barriers. BioEssays 2005;27:356–365.
108. Furuse M, Tsukita S. Claudins in occludin junctions of humans and flies. Trends Cell Biol 2006;16:181–188.
109. González-Mariscal L, Betanzos A, Nava P, et al. Tight junction proteins. Prog Biophys Mol Biol 2003;81:1–44.
110. Van Itallie CM, Fanning AS, Anderson JM. Reversal of charge selectivity in cation or anion-selective epithelial lines by expression of different claudins. Am J Physiol Renal Physiol 2003;285: F1078–F1084.
111. Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. Annu Rev Physiol 2006;68:403–429.
112. Lourenço SV, Coutinho-Camillo CM, Buim MEC, et al. Human salivary gland branching morphogenesis: morphological location of claudins and its parallel relation with developmental stages revealed by expression of cytoskeleton and secretion markers. Histochem Cell Biol 2007;128:361–369.
113. Michikawa H, Fujita-Yoshigaki J, Sugiya H. Enhancement of barrier function by overexpression of claudin-4 in tight junctions of submandibular gland cells. Cell Tissue Res 2008;334:255–264.
114. Peppi M, Ghabriel MN. Tissue-specific expression of the tight junction proteins claudins and occludin in the rat salivary glands. J Anat 2004;205:257–266
115. Hashizume A, Ueno T, Furuse M, et al. Expression patterns of claudin family of tight junction membrane proteins in developing mouse submandibular gland. Dev Dyn 2004;231:425–431.
116. Kriegs JO, Homann V, Kinne-Saffran E, et al. Identification and subcellular localization of paracellin-1 (claudin-16) in human salivary glands. Histochem Cell Biol 2007;128:45–53.
117. Aframian DJ, Tran SD, Cukierman E, et al. Absence of tight junction formation in an allogeneic graft cell line used for developing an engineered artificial salivary gland. Tissue Eng 2002;8:871–878.
118. Qi B, Fujita-Yoshigaki J, Michikawa H, et al. Differences in claudin synthesis in primary cultures of acinar cells from rat salivary gland are correlated with the specific three-dimensional organization of the cells. Cell Tissue Res 2007;329:59–70.
119. Pade V, Stavchansky S. Estimation of the relative contribution of the transcellular and paracellular pathay to the transport of passively absorbed drugs in the Caco-2 cell culture model. Pharm Res 1997;14:1210–1215.
120. Bourdet DL, Pollack GM, Thakker DR. Intestinal absorptive transport of the hydrophilic cation ranitidine: A kinetic modeling approach to elucidate the role of uptake and efflux transporters and paracellular vs. transcellular transport in Caco-2 cells. Pharm Res 2006;23:1178–1187.
121. Adson A, Burton PS. Raub TJ, et al. Passive diffusion of weak organic electrolytes across Caco-2 cell monolyaers: Uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers. J Pharm Sci 1995;84:1197–1204.
122. He YL, Murby S, Warhurst G, et al. Species differences in size discrimination in the paracellular pathway reflected by oral bioavailability of poly(ethylene glycol) and D-peptides. J Pharm Sci 1998;87:626–633.
123. Knipp GT, Ho NF, Barsuhn CL, et al. Paracellular diffusion in Caco-2 cell monolayers: effect of perturbation on the transport of hydrophilic compounds that vary in charge and size. J Pharm Sci 1997;86:1105–1110.
124. Murakami M, Shachar-Hill B, Steward MC, et al. The paracellular component of water flow in the rat submandibular salivary gland. J Physiol 2001;537:899–906.

125. Watson CJ, Rowland M, Warhurst G. Functional modeling of tight junctions in intestinal cell monolayers using polyethylene glycol oligomers. Am J Physiol Cell Phyiol 2001;281:C388–C397.
126. Van Itallie CM, Holmes J, Bridges A, et al. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. J Cell Sci 2008;121:208–305.
127. Artursson P, Ungell AL, Lofroth JE. Selective paracellular permeability in two models of intestinal absorption: Cultured monolayers of human intestinal epithelial cells and rat intestinal segments. Pharm Res 1993;10:1123–1129.
128. Case RM, Cook DI, Hunter M, et al. Transepithelial transport of nonelectrolytes in the rabbit mandibular salivary gland. J Membr Biol 1985;84:239–248.
129. Hou J, Gomes AS, Paul DL, Goodenough DA. Study of claudin function by RNA interference. J Biol Chem 2006;281:36117–36123.
130. Rosenthal R, Milatz S, Krug SM, et al. Claudin-2, a component of the tight junction, forms a paracellular water channel. J Cell Sci 2010;123:1913–1921.
131. Borovac J, Barker RS, Rievaj J, et al. Claudin-4 forms a paracellular barrier, revealing the interdependence of claudin expression in the loose epithelial cell culture model opossum kidney cells. Am J Physiol Cell Physiol 2012;303:C1278–C1291.
132. Milatz S, Krug SM, Rosenthal R, et al. Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. Biochim Biophys Acta 2010;1798:2048–2057.
133. Mitchell LA, Overgaard CE, Ward C, et al. Differential effects of claudin-3 and claudin-4 on alveolar epithelial barrier function. Am J Physiol Lung Cell Mol Physiol 2011;301:L40–L49.
134. Kawedia JD, Nieman ML, Boivin GP, et al. Interaction between transcellular and paracellular water transport pathways through Aquaporin 5 and the tight junction complex. Proc Natl Acad Sci 2007;104: 3621–3626.
135. Alexandre MD, Lu Q, Chen YH. Overexpression of claudin-7 decreases the paracellular Cl- conductance and increases the paracellular Na^+ conductance in LLC-PK1 cells. J Cell Sci 2005;118: 2683–2693.
136. Avdeef A. Leakiness and size exclusion of paracellular channels in cultured epithelial cell monolayers-interlaboratory comparison. Pharm Res 2010;27:480–489.
137. Simson JAV, Dom RM. The use of lead as an ionic tracer for investigating routes of passive fluid transfer. J Histochem Cytochem 1983;31:675–683.
138. Hashimoto S, Murakami M, Kanaseki T, et al. Morphological and functional changes in cell junctions during secretory stimulation in the perfused rat submandibular gland. Eur J Morphol 2003;41:35–39.
139. Segawa A, Yamashina S, Murakami M. Visualization of 'water secretion' by confocal microscopy in rat salivary glands: possible distinction of para- and transcellular pathway. Eur J Morphol 2002;40:241–246.
140. Mitsui R, Fujita-Yoshigaki J, Narita T, et al. Maintenance of paracellular barrier function by insulin-like growth factor-1 in submandibular gland cells. Arch Oral Biol 2010;55:963–969.
141. Tomita M, Hotta Y, Ohkubo R, et al. Polarized transport was observed not in hydrophilic compounds but in detran in Caco-2 cell monolayers. Biol Pharm Bull 1999;22:330–331.
142. Ohkubo R, Tomita M, Hotta Y, et al. Comparative study of flux of FITC-labeled dextran 4000 on normal (iso)- and hyper-osmolarity in basal side in Caco-2 cell monolayers. Drug Metab Pharmacokin 2003;18:404–408.
143. Bradbury NA, Bridges RJ. Endocytosis is regulated by protein kinase A, but not protein kinase C in a secretory epithelial cell line. Biochem Biophys Res Comm 1992;184:1173–1180.
144. Ivanov AI, Nusrat A, Parkos CA. Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. Mol Biol Cell 2004;15:176–188.
145. Shurety W, Stewart NL, Stow JL. Fluid-phase markers in the basolateral endocytic pathway accumulate in response to the actin assembly-promoting drug jasplakinolide. Mol Biol Cell 1998;9:957–975.
146. Matsukawa Y, Lee VH, Crandall ED, et al. Size-dependent dextran transport across rat alveolar epithelial cell monolayers. Pharm Sci 1997;86:305–309.
147. Krugliak P, Hollander D, Ma TY, et al. Mechanisms of poly(ethylene glycol) 400 permeability of perfused rat intestine. Gastroenterology 1989;97:1164-1170.
148. Hollander D, Koyama S, Dadufalza V, et al. Poly(ethylene glycol) 900 permeability of rat intestinal and colonic segments in vivo and brush border membrane in vitro. J Lab Clin Med 1989;113:505–515.
149. Van Itallie CM, Anderson JM. Measuring size-dependent permeability of the tight junction using PEG profiling. Methods Mol Biol 2011;762:1–11.
150. Iqbal TH, Hollander D, Krugliak P, et al. PEG 400, a hydrophilic molecular probe for measureing intestinal permeability. Gastroenterology 1990;98:39–46.
151. Maxton DG, Bjarnason I, Reynolds AP, et al. Lactulose, ^{51}Cr-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethyleneglycol 400 as probe markers for assessment in vivo of human intestinal permeability. J Clin Sci 1986;71:71–80.
152. Brayden DJ, Bzik VA, Lewis AL, et al. CriticalSorb^{TM} promotes permeation of flux markers across isolated rat intestinal mucosae and Caco-2 monolayers. Pharm Res 2012;29:2543–2554.
153. Linnankoski J, Mäkelä J, Palmgren J, et al. Paracellular porosity and pore size of the human intestinal epithelium in tissue and cell culture models. J Pharm Sci 2010;99:2166–2175.
154. Shachar-Hill B, Hill AE. Paracellular fluid transport by epithelia. Inter Rev Cytol 2002;215:319-350.
155. Howorth AJ, Case RM, Steward MC. Effects of acetylcholine and forskolin on the non-electrolyte permeability of the perfused rabbit mandibular gland. Pflugers Arch 1987;408:209–214.
156. Steward MC. Paracellular non-electrolyte permeation during fluid transport across rabbit gall-bladder epithelium. J Physiol 1982;322:419–439.
157. Nakahari T, Imai Y. Transient swelling of salivary acinus induced by acetylcholine stimulation: Water secretion pathway in rat submandibular gland. J Membr Biol 1998;161:287–296.
158. Knipp GT, Vander Velde DG, Siahaan TJ, et al. The effect of beta-turn structure on the passive diffusion of peptides across Caco-2 cell monolayers. Pharm Res 1997;14:1332–1340.
159. Tavelin S, Taipalensuu J, Söderberg L, et al. Prediction of the oral absorption of low-permeability drugs using small intestine-like 2/4/A1 cell monolayers. Pharm Res 2003;20:397–405.
160. Seki T, Harada S, Hosoya O, et al. Evaluation of the establishment of a tight junction in Caco-2 cell monolayers using a pore permeation model involving two different sizes. Biol Pharm Bull 2008;31:163–166.
161. Kataoka M, Iwai K, Masaoka Y, et al. Scale-up of in vitro permeation assay data to human intestinal permeability using pore theory. Int J Pharm 2011;414:69–76.
162. Watson CJ, Hoare CJ, Garrod DR, et al. Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. J Cell Sci 2005; 118:5221–5230.
163. Tang VW, Goodenough DA. Paracellular ion channel at the tight junction. Biophys J 2003;84:1660–1673.
164. Guo P, Weinstein AM, Weinbaum S. A dual-pathway ultrastructual model for the tight junction of rat proximal tubule epithelium. Am J Physiol Renal Physiol 2003;285:F241–F257.
165. Orci L, Humbert F, Brown N, et al. Membrane ultrastructure in urinary tubules. Int Rev Cytol 1981;73:183–242.
166. Hasegawa H, Fujita H, Katoh H, et al. Opposite regulation of transepithelial electrical resistance and paracellular permeability by Rho in Madin-Darby canine kidney cells. J Biol Chem 1999;274: 20982–20988.

167. Moreno JH. Routes of nonelectrolytepermeability in gallbladder. Effects of 2,4,6-triaminopyrimidinium (TAP). J Gen Physiol 1975;66:118–128.
168. Garrett JR, Parsons PA. Movement of horseradish peroxide in rabbit submandibular glands after ductal injection. Histochem J 1976;8:177–189.
169. Mazariegos MR, Tice LW, Hand AR. Alteration of tight junctional permeability in the rat parotid gland after isoproterenol stimulation. J Cell Biol 1984;98:1865–1877.
170. Mazariegos MR, Hand AR. Regulation of tight junctional permeability in the rat parotid gland by autonomic agonists. J Dent Res 1984;63:1102–1107.
171. Takai N, Uchihashi K, Miyao H, et al. Chorda-evoked opening of tight junctions in rat submandibular salivary acini demonstrated by microperoxidase. Arch Oral Biol 1995;40:1077–1080.
172. Oliver C, Hand AR. Uptake and fate of lumenally administered horseradish peroxidase in resting and isoproterenol-stimulated rat parotid acinar cells. J Cell Biol 1978;76:207–229.
173. Kuijpers GA, Van Nooy IG, Vossen ME, et al. Tight junctional permeability of the resting and carbachol stimulated exocrine rabbit pancreas. Histochemistry 1985;83:257–264.
174. Matsukawa Y, Lee VH, Crandall ED, et al. Size-dependent dextran transport across rat alveolar epithelial cell monolayers. J Pharm Sci 1997;86:305–309.
175. Hu XP, Weinbaum S. A new view of Starling's hypothesis at the microstructural level. Microvasc Res 1999;58:281–304.
176. Matsuzaki T, Suzuki T, Koyama H, et al. Aquaporin-5 (AQP5), a water channel protein in the rat salivary and lacrimal glands: Immunolocation and effect of secretory stimulation. Cell Tissue Res 1999;95:513–521.
177. Ishikawa Y, Skowronski NT, Iboue N, et al. 1 Adrenoceptor-induced trafficking of aquaporin-5 to the apical plasma membrane of rat parotid cells. Biochem Biophys Res Comm 1999;265:94–100.
178. Crestz V, Kwon TH, Hurley PT, et al. Identification and localization of aquaporin water channels in human salivary glands. Am J Physol Gastraintest Liver Physiol 2001;281:G247–G254.
179. Ma T, Song Y, Gillespie A, et al. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem 1999;274:20071–20074.
180. Krane CM, Fortner CN, Hand AR, et al. Aquaporin 5-deficient mouse lungs are hyperresponsive to cholinergic stimulation. Proc Natl Acad Sci USA 2001;98:14114–14119.
181. Zeuthen T. Water-transporting proteins. J Membr Biol 2010;234:57–73.
182. Zeuthen T, MacAulay N. Passive water transport in biological pores. Int Rev Cytol 2002;215:203–230.
183. Zeuthen T, MacAulay N. Cotransporters as molecular water pumps. Int Rev Cytol 2002;215:259–284.
184. Murakami M, Murdiastuti K, Hosoi K, et al. AQP and the control of fluid transport in a salivary gland. J Membr Biol 2006;210:91–103.
185. Hill AE, Shachar-Hill B, Shachar-Hill Y. What are aquaporins for? J Membr Biol 2004;197:1–32.
186. Hill AE, Shachar-Hill B. A new approach to epithelial isotonic fluid transport: An osmosensor feedback model. J Membr Biol 2006;210:77–90.
187. Gin E, Crampin EJ, Brown DA, et al. A mathematical model of fluid secretion from a parotid acinar cell. J Theoret Biol 2007;248:64–80.
188. Fischbarg J. Fluid transport across leaky epithelia: Central role of the tight junction and supporting role of aquaporins. Physiol Rev 2010;90:1271–1290.
189. Palk L, Sneyd J, Shuttleworth TJ, et al. A dynamic model of saliva secretion. J Theoret Biol 2010;266:625–640.
190. Maclaren OJ, Sneyd J, Crampin EJ. Efficiency of primary saliva secretion: A analysis of parameter dependence in dynamic single-cell and acinus models, with application to aquaporin knockout studies. J Membr Biol 2012;245:29–50.
191. Murakami M, Hashimoto S, Wei M, et al. Morpho-physiological approach to the paracellular route for salivary secretion by isolated perfused submandibular gland. J Med Invest 2009;56 Suppl:322–324.
192. Nakahari T, Yoshida H, Imai Y. Transepithelial fluid shift generated by osmolarity gradients in unstimulated perfused rat submandibular glands. Exp Physiol 1996;81:767–779.
193. Tanrattana C, Charoenphandu N, Limlomwongse L, et al. Prolzctin directly stimulated the solvent drag-induced calcium transport in the duodenum of female rats. Biochim Biophys Acta 2004;1665:81–91.
194. Charoenphandu N, Tudpor K, Pulsook N, et al. Chronic metabolic acidosis stimulated transcellular and solvent drag-induced calcium transport in the duodenum of female rats. Am J Physiol Gastrointest Liver Physiol 2006;291:G446–G455.
195. Tudpor K, Teeraporpuntakit J, Jantarajit W, et al. 1,25-Dihydroxyvitamin D3 rapidly stimulates the solvent drag-induced paracellular calcium transport in the duodenum of female rates. J Physiol Sci 2008;58:297–307.
196. D'Souza T, Agarwal R, Morin PJ. Phosphorylation of claudin-3 at threonine 192 by cAMP-dependent protein kinase regulates tight junction barrier function in ovarian cancer cells. J Biol Chem 2005;280:26233–26240.
197. Soma T, Chiba H, Kato-Mori Y, et al. Thr(207) of claudin-5 is involved in size-selective loosening of the endothelial barrier by cyclic AMP. Exp Cell Res 2004;300:202–212.
198. Ikari A, Matsumoto S, Harada H, et al. Phosphorylation of paracellin-1 at Ser217 by protein kinase A is essential for localization in tight junctions. J Cell Sci 2006;119:1781–1789.
199. Sakakibara A, Furuse M, Saitou M. Possible involvement of phosphorylation of occludin in tight junction formation. J Cell Biol 1997;137:1393–1401.
200. Blasig IE, Bellmann C, Cording J, et al. Occludin protein family: oxidative stress and reducing conditions. Antioxid Redox Signal 2011;15:1195–1219.
201. Rao R. Occludin phosphorylation in regulation of epithelial tight junctions. Ann NY Acad Sci 2009;1165:62–68.
202. Shen L. Tight junctions on the move: molecular mechanisms for epithelial barrier regulation. Ann NY Acad Sci 2012;1258:9–18.
203. Raleigh DR, Boe DM, Yu D, et al. Occludin S408 phosphorylation regulates tight junction protein interaction and barrier function. J Cell Biol 2011;193:565–582.
204. Ozaki H, Ishii K, Arai H, et al. Junctional adhesion molecule (JAM) is phosphorylated by protein C upon platelet activation. Biochem Biophys Res Commun 2000;276:873–878.
205. Iden S, Misselwitz S, Peddibhotla SS, et al. aPKC phosphorylates JAM-A at Ser285 to promote cell contact maturation and tight junction formation. J Cell Biol 2012;196:623–639.
206. Rodgers LS, Fanning AS. Regulation of epithelial permeability by the actin cytoskeleton. Cytoskeleton 2011;68:653–660.
207. Shen L, Weber CR, Turner JR. The tight junction protein complex undergoes rapid and continuous molecular remodeling at steady state. J Cell Biol 2008;181:683–695.
208. Ekström J. Role of nonadrenergic, noncholinergic autonomic transmitters in salivary glandular activities in vivo. In: Garrett JR, Ekström J, Anderson LC (ed). Neural Mechanisms of Salivary Secretion. vol 6. Karger, Basel, 1999:94–130.
209. Liu X-B, Sun X, Mörk A-C, et al. Characterization of the calcium signaling system in the submandibular cell line SMG-C6. Proc Soc Exp Biol Med 2000;225:211–220.
210. Liu X-B, Mörk A-C, Sun X, et al. Regulation of Ca^{2+} signals in a parotid cell line Par-C5. Arch Oral Biol 2001;46:1141–1149.
211. Castro R, Barlow-Walden L, Woodson T, et al. Ion transport in an immortalized rat submandibular cell line SMG-C6. Proc Soc Exp Biol Med 2000;225:39–48.
212. Kawabe H, Takai N. Autonomic regulation of tight junctional permeability in the rat submandibular gland. J Osaka Dent Univ 1990;24:121–134.

213. Garrett JR, Klinger AH, Parsons PA. Permeability of canine submandibular glands to blood-borne horseradish peroxidase during parasympathetic secretion. Quart J Exp Physiol 1982;67:31–39.
214. Steward MC, Seo Y, Rawlings JM, et al. Water permeability of acinar cell membranes in the isolated perfused rabbit mandibular salivary gland. J Physiol 1990;431:571–583.
215. Segawa A. Tight junctional permeability in living cells: dynamic changes directly visualized by confocal laser microscopy. J Electron Microsc 1994;43:290–298.
216. Takai N, Uchihashi K, Yoshida Y, et al. Permeability increase in rat submandibular gland by glandular substance P. Proc Finn Dent Soc 1989;85:339–344.
217. Tani Y, Uchihashi K. Distribution of anionic sites during increasing tight junctional permeability in the rat submandibular gland. J Osaka Dent Univ 1993;27:23–35.
218. Qi B, Narita T, Satoh K, et al. Characterizatics of neurokinin A-induced salivary fluid secretion in perfused rat submandibular gland. Arch Oral Biol 2010;55:737–744.
219. Lacaz-Vieira F, Marques MM. Pulses of Ca^{2+} and the dynamics of tight junction opening and closing. J Membr Biol 2003;196: 117–127.
220. Yu ASL, Cheng MH, Coalson RD. Calcium inhibits paracellular sodium conductance through claudin-2 by competitive binding. J Biol Chem 2010;285:37060–37069.
221. Mörk AC, Zhang GH, Martinez JR. Modulation of Ca^{2+} mobilization by protein kinase C in rat submandibular acinar cells. J Cell Biochem 1999;72:47–55.
222. Clarke H, Marano CW, Soler AP. Modification of tight junction function by protein kinase C isoforms. Adv Drug Del Rev 2000;41: 283–301.
223. Lacaz-Vieira F. Tight junction dynamics: oscillations and the role of protein kinase C. J Membr Biol 2000;178:151–161.
224. Inukai Y, Ikeda R, Aiyama S. Effect of glucocorticoid on the differentiation and development of terminal tubules in the fetal rat submandibular gland. Cell Tiss Org 2008;187:233–242.
225. Sekiyama A, Gon Y, Terakado M, et al. Glucocorticoids enhance airway epithelial barrier integrity. Int Immunopharmacol 2012;12: 350–357.
226. Stelwagen K, McFadden HA, Demmer J. Prolactin, alone or in combination with glucocorticoids, enhances tight junction formation and expression of the tight junction protein occludin in mammary cells. Mol Cell Endocrinol 1999;156:55–61.
227. Boivin MA, Ye D, Kennedy JC, et al. Mechanism of glucocorticoid regulation of the intestinal tight junction barrier. Am J Physiol Gastrointest Liver Physiol 2007;292:G590–G598.
228. Kimura K, Teranishi S, Kawamoto K, et al. Protective effect of dexamethasone against hypoxia-induced disruption of barrier function in human corneal epithelial cells. Exp Eye Res 2011;92:388–393.
229. Peixoto EB, Collares-Buzato CB. Modulation of the epithelial barrier by dexamethasone and prolactin in cultured Madin-Darby canine kidney (MDCK) cells. Cell Biol Int 2006;30:101–113.
230. Vasquez MM, Mustafa SB, Choudary A, et al. Regulation of epithelial Na^+ channel (ENaC) in the salivary cell line SMG-C6. Exp Biol Med 2009;234:522–531.
231. Beal AM. Effects of flow rate, duration of stimulation and mineralocorticoids on the electrolyte concentrations of mandibular saliva from the red kangaroo (Macropus rufus). J Exp Biol 1986;126:315–339.
232. Riad F, Lefaivre J, Tournaire C, et al. Aldosterone regulates salivary sodium secretion in cattle. J Endocrinol 1986;108:405–411.
233. Hoffmann B, Nagel I, Clauss W. Aldosterone regulates paracellular pathway resistance in rabbit distal colon. J Comp Physiol B 1990;160:381–388.
234. Moretó M, Cristià E, Pérez-Bosque A, et al. Aldosterone reduces crypt colon permeability during low-sodium adaptation. J Membr Biol 2005;206:43–51.
235. Amasheh S, Milatz S, Krug SM, et al. Na^+ absorption defends from paracellular back-leakage by claudin-8 upregulation. Biochem Biophys Res Commun. 2009;378:45–50.
236. Le Moellic C, Boulkroun S, González-Nunez D, et al. Aldosterone and tight junctions: modulation of claudin-4 phosphorylation in renal collecting duct cells. Am J Physiol Cell Physiol 2005;289: C1513–C1521.
237. Porola P, Laine M, Virkki L, et al. The influence of sex steroids on Sjögren's syndrome. Ann N Y Acad Sci 2007;1108:426–432.
238. Porola P, Straub RH, Virkki LM, et al. Failure of oral DHEA treatment to increase local salivary androgen outputs of female patients with Sjögren's syndrome. Scand J Rheumatol 2011;40:387–390.
239. McCabe MJ, Allan CM, Foo CF, et al. Androgen initiates Sertoli cell tight junction formation in the hypogonadal (hpg) mouse. Biol Reprod 2012;87:1–8.
240. Kolasa A, Marchlewicz M, Wenda-Różewicka L, et al. DHT deficiency perturbs the integrity of the rat seminiferous epithelium by disrupting tight and adherens junctions. Histochem Cytobiol 2011;49:62–71.
241. Stumpf WE, Hayakawa N. Salivary glands epithelial and myoepithelial cells are major vitamin D targets. Eur J Drug Metab Pharmacokinet 2007;32:123–129.
242. Glijer B, Peterfy C, Tenenhouse A. The effect of vitamin D deficiency on secretion of saliva by rat parotid gland in vivo. J Physiol 1985;363:323–334.
243. Peterfy C, Tenenhouse A, Yu E. Vitamin D and parotid gland function in the rat. J Physiol 1988;398:1–13.
244. Fujita H, Sugimoto K, Inatomi S, et al. Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca^{2+} absorption between enterocytes. Mol Biol Cell 2008;19:1912–1921.
245. Khristakos S. Mechanism of action of 1,25-dihydroxyvitamin D3 on intestinal calcium absorption. Rev Endor Metab Disord 2012;13:39–44.
246. Chirayath MV, Gajdzik L, Hulla W, et al. Vitamin D increases tight-junction conductance and paracellular Ca^{2+} transport in Caco-2 cell cultures. Am J Physiol 1998;274:G389–G396.
247. Singh AB, Harris RC. Epidermal growth factor receptor activation differentially regulates claudin expression and enhances transepithelial resistance in Madin-Darby canine kidney cells. J Biol Chem 2004;279:3543–3552.
248. Ikari A, Atomi K, Takiguchi A, et al. Epidermal growth factor increases claudin-4 expression mediated by Sp1 elevation in MDCK cells. Biochem Biophys Res Commun 2009;384:306–310.
249. Ikari A, Takiguchi A, Atomi K, et al. Epidermal growth factor increases clathrin-dependent endocytosis and degradation of claudin-2 protein in MDCK II cells. J Cell Physiol. 2011;226: 2448–2456.
250. Samak G, Aggarwal S, Rao RK. ERK is involved in EGF-mediated protection of tight junctions, but not adherens junctions, in acetaldehyde-treated Caco-2 cell monolayers. Am J Physiol Gastrointest Liver Physiol 2011;301:G50–G59.
251. Guntaka SR, Samak G, Seth A, et al. Epidermal growth factor protects the apical junctional complexes from hydrogen peroxide in bile duct epithelium. Lab Invest 2011;91:1396–1409.
252. Kerr M, Lee A, Wang PL, et al. Detection of insulin and insulin-like growth factors I and II in saliva and potential synthesis in the salivary glands of mice. Effect of Type 1 diabetes mellitus. Biochem Pharmacol 1995;49:1521–1531.
253. Amano O, Iseki S. Expression, localization and developmental regulation of insulin-like growth factor I mRNA in rat submandibular gland. Arch Oral Biol 1993;38:671–677.
254. Nakamura Y, Yamamoto M, Sakamoto K, et al. Growth factors, extracellular matrix components and cell adhesion molecules in Warthin's tumor. J Oral Pathol Med 2001;30:290–295.
255. Roescher N, Tak PP, Illei GG. Cytokines in Sjögren's syndrome. Oral Dis 2009;15:519–526.

256. Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. Biochim Biophys Acta 2009;1788:864–871.
257. Al-Sadi R, Boivin M, Ma T. Mechanism of cytokine modulation of epithelial tight junction barrier. Front Biosci 2009;14:2765–2778.
258. Bikker A, Van Woerkom JM, Kruize AA, et al. Increased expression of interleukin-7 in labial salivary glands of patients with primary Sjögren's syndrome correlates with increased inflammation. Arth Rheum 2010;62:969–977.
259. Petecchia L, Sabatini F, Usai C, et al. Cytokines induce tight junction disassembly in airway cells via an EGFR-dependent MAPK/ERK1/2-pathway. Lab Invest 2012;92:1140–1148.
260. Norheim KB, Jonsson G, Harboe E, et al. Oxidative stress, as measured by protein oxidation, is increased in primary Sjögren's syndrome. Free Rad Res 2012;46:141–146.
261. Pagano G, Castello G, Pallardó FV. Sjögren's syndrome-associated oxidative stress and mitochondrial dysfunction: Prospects for chemoprevention trials. Free Rad Res 2013;47:71–73.
262. Basuroy S, Seth A, Elias B, et al. MAPK interacts with occludin and mediates EGF-induced prevention of tight junction disruption by hydrogen peroxide. Biochem J 2006;393:69–77.
263. Gonzalez JE, DiGeronimo RJ, Arthur DE, et al. Remodeling of the tight junction during recovery from exposure to hydrogen peroxide in kidney epithelial cells. Free Rad Biol Med 2009;47:1561–1569.
264. Kiely B, Feldman G, Ryan MP. Modulation of renal epithelial barrier function by mitogen-activated protein kinases (MAPKs): mechanism of cyclosporine A induced increase in transepithelial resistance. Kidney Int 2003;63:908–916.
265. Lipschutz JH, Li S, Arisco A, et al. Extracellular signal-regulated kinases 1/2 control claudin-2 expression in Madin-Darby canine kidney strain I and II cells. J Biol Chem 2005;280:3780–3788.