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REVIEW

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Regulation of the blood–biliary barrier: interaction between gap and tight junctions in hepatocytes

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Abstract Hepatocytes tightly connect with each other by intercellular junctions to form liver cell plates. The junctions composed of gap, tight, and adherens junctions and desmosomes concentrate around bile canaliculi. In particular, tight junctions serve as a barrier to keep bile in bile canaliculi away from the blood circulation. Thus, it is very reasonable to call tight junctions of hepatocytes the blood–biliary barrier. On the other hand, gap junctions of hepatocytes are considered to enable ordered contraction of bile canaculi from centrizonal to periportal hepatocytes by their function of intercellular communication. Gap and tight junctions may thus play a crucial role in bile secretion, one of the most differentiated functions of the liver. In intrahepatic cholestasis, a common pathological condition of the liver, downregulation of gap and tight junctional functions is seen, which results in impaired intercellular communication and in leaky tight junctions. Although the changes in gap and tight junctions had been considered to be independent of each other, recent findings that the tight junction-associated proteins ZO-1 and occludin bind to connexins indicate the possibility of either coordinate or reciprocal regulation of macromolecular complexes containing gap- and tight-junction proteins. In this review, we introduce the interaction and regulation between gap and tight junctions of hepatocytes in vitro and discuss the regulatory mechanisms of the "blood–biliary barrier" to study the molecular pathogenesis of cholestasis.

Key words Gap junctions · Tight junctions · Blood–biliary barrier · Actin filament · Hepatocytes · Cholestasis

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Introduction

Bile secretion, one of the most differentiated functions of the liver, essentially requires gap and tight junctions. Because gap junctions regulate direct intercellular communication and tight junctions strictly seal the bile canaliculi, bile flow is ensured without leakage of bile from the centrizonal to the periportal zone and then to bile ducts in a highly ordered manner. In humans and animals, most cholestatic liver diseases are associated with profound changes in the pericanalicular region (Fig. 1). Cholestatic hepatocytes show downregulation of gap and tight junctional functions, resulting in impaired intercellular communication of gap junctions[1,2] and in deteriorated barrier function of tight junctions.[3–5] Depolymerization of actin-myosin bundles also results in the loss of canalicular membrane tone and failure to contract.[6,7] Finally, dilated bile canaliculi are observed in cholestatic hepatocytes (Fig. 1D).

In this article, we summarize the recent findings on gap junctions and tight junctions, particularly from the point of view of linkage of these junctions with actin filaments in hepatocytes in vivo and in vitro, to elucidate the detailed molecular pathogenesis of cholestasis.

Structure of gap and tight junctions in the liver

Thin sections and freeze-fracture images of the junctions between dissociated hepatocytes reveal structures that are similar to those seen in vivo (Fig. 2). In vertebrates, tight junctions play a central role in regulating the movement of solutes, ions, and water through the extracellular space in epithelial or endothelial sheets. Tight junctions between hepatocytes are found surrounding the bile canaliculi, where they seal the paracellular spaces between hepatocytes and maintain cellular polarity. By contrast, gap junctions control cell-to-cell movement of solutes, ions, and water, providing an intercellular pathway not accessible to dilution by extracellular fluid. In the hepatic

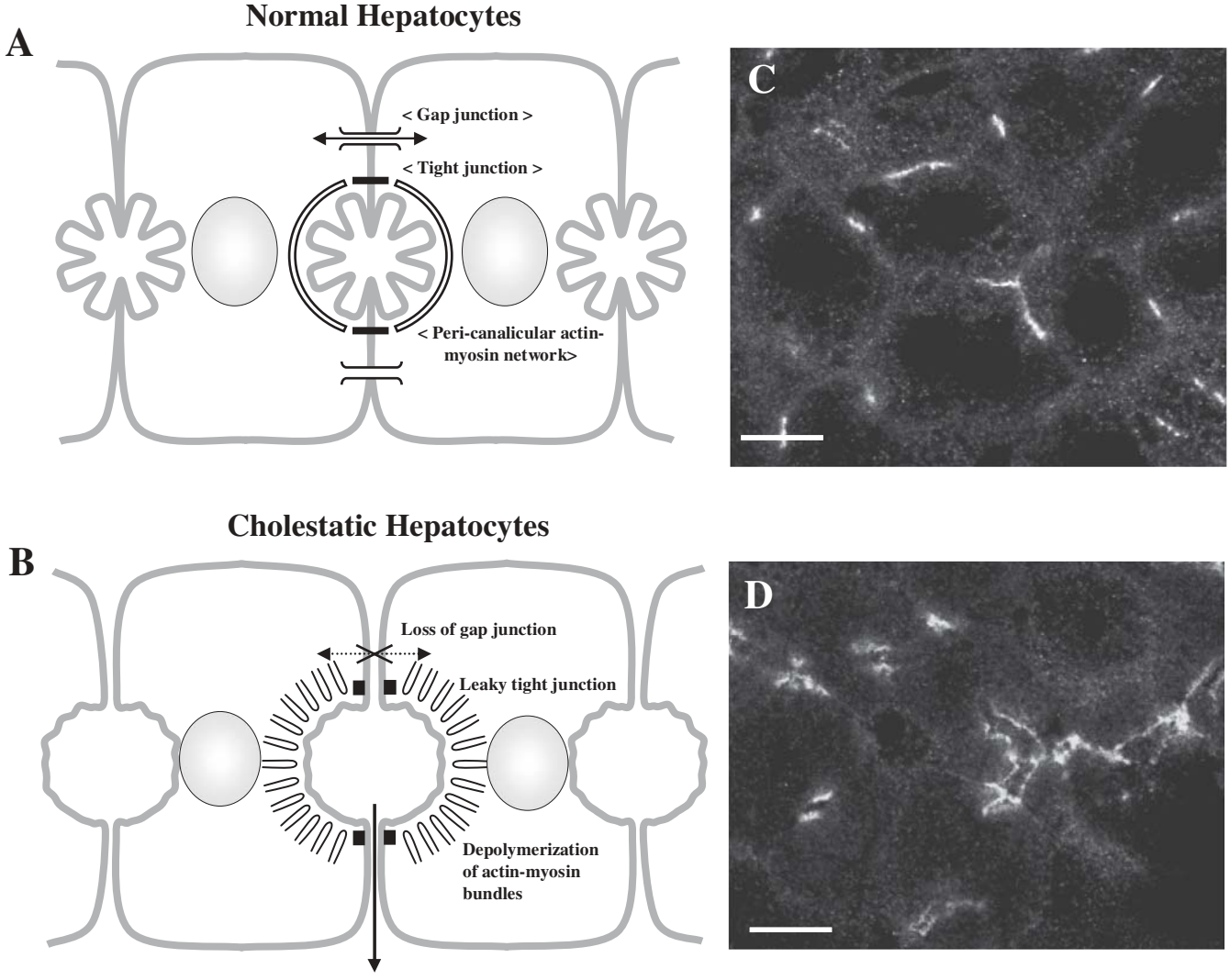

Fig. 1. Changes of gap and tight junctions and cytoskeleton in cholestatic hepatocytes. Normal hepatocytes (**A**) have gap junctions, which facilitate intercellular communication, tight junctions, which seal the canalicular lumen, and a pericanalicular actin-myosin network that facilitates the flow of bile from pericentral to periportal lobular regions. Cholestatic hepatocytes (**B**) show loss of gap junctions, leaky tight junctions, and depolymerization of actin-myosin bundles. **C,D** Fluorescent immunostaining for ZO-1 in normal rat liver (**C**) and common bile duct-ligated rat liver (**D**). At 48 h after common bile duct ligation, dilated bile canaliculi are observed. *Bars* 10 μm

acinus, bile flow in hepatocyte canaliculi is thought to require the organized and periodic contraction of bile canaliculi, where cell-to-cell spread of contraction occurs through signaling via gap-junction channels.[8] In the perfused rat liver, bile secretion induced by glucagon or vasopressin has been shown to be modulated by gap-junction communication.[9]

Gap junctions occupy a large fraction, as much as 3%, of the total surface area of hepatocytes.[10,11] In freeze-fracture images, gap junctions of liver and hepatocyte cell pairs are recognizable as arrays or plaques of approximately 8- to 9-nm intramembranous particles present in the P-fracture face in vertebrate tissues; complementary pits appear on the E-fracture face. These plaques are generally round or oval and can be quite large in hepatocytes (Fig. 2C), commonly exceeding 1 μm in diameter and containing more than 10 000 particles.

The ultrastructure of tight junctions is quite different from that of gap junctions. In freeze fractures, tight junctions appear as a set of continuous, anastomosing strands in the P-fracture face (Fig. 2B), with complementary grooves in the E-face; in thin section, they appear as very close membrane appositions.[12,13] The role of tight junctions is to provide a high-resistance barrier to leakage of water and solutes into and from the bile canaliculus (the resistance to current flow has been measured as approximately 50 Kohms by insertion of a microelectrode into the canalicular space[14]). The number of tight junctional strands encircling the bile canaliculus is normally high, indicating a tight permeability barrier.[15] As a consequence, the composition of the canalicular fluid can be quite different from that of the other pericellular fluid, allowing the accumulation of high concentrations of preferentially secreted organic anions within this intercellular compartment.[14,16]

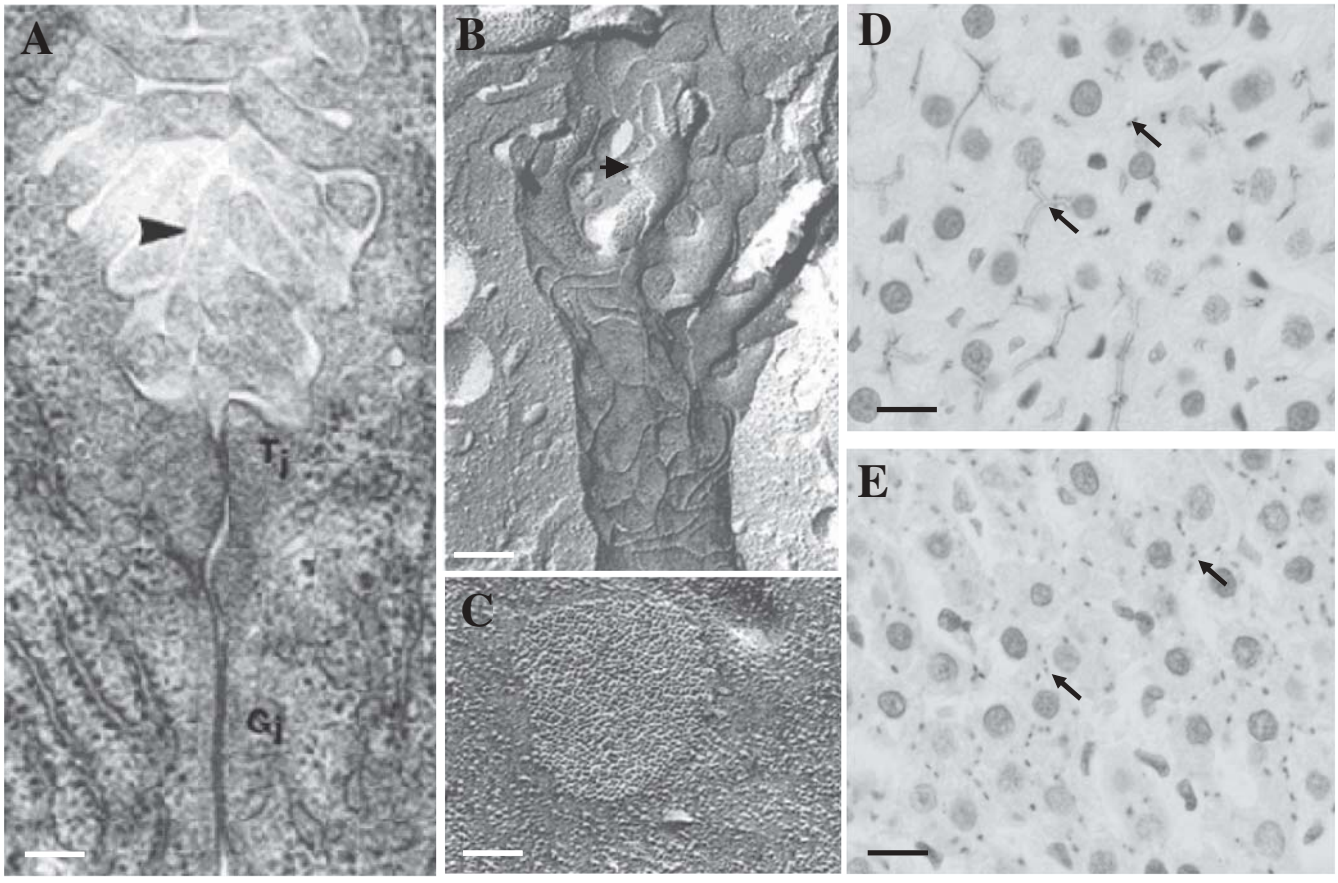

Fig. 2. Fine structure of appositional membranes between rat hepatocytes (**A**, thin section; **B,C**, freeze-fracture; **D,E**, immunostaining of claudin-1 and Cx32 using paraffin sections). **A** Thin-section micrograph illustrates tight junctions (*Tj*) sealing the canaliculus (*arrowhead*) and a large gap junction (*Gj*) below it. **B** Below the bile canaliculi (*arrowhead*), tight-junction webs seen in this freeze-fracture electron micrograph seal off the canalicular surfaces from the rest of the appositional membrane. **C** Freeze-fracture views show larger gap junctions below and in isolation from tight junctional strands. (From ref. 1, with permission) *Bars* **A–C** 0.1 nm; **D,E** 20 μm

Molecular components and regulation of gap and tight junctions

Hepatic gap junctions

Cx32 and Cx26, the components of hepatocyte gap junctions (Fig. 2E), are also found in a variety of other cell types (for review, see ref. 17); moreover, another gap junction protein, Cx43 (or α1), is prominent between other liver cell types, including Ito cells and endothelium. The connexin gene family now comprises at least 20 proteins in vertebrates;[18] connexins are apparently absent in invertebrates, where gap junction channels are formed by a separate gene family encoding innexin proteins.[19]

Most cells express more than one type of connexin, leading to the possibility that both homomeric and heteromeric connexons may exist in vivo. The existence of heteromeric connexons in the liver has been supported by biochemical experiments that have fractionated detergent-solubilized gap junctions[20] and the electrophysiological properties of hepatocytes isolated from Cx32-deficient and wild-type mice.[21] Experiments examining the movement of ions and dyes between cells coupled by different connexins have revealed that there are connexin-dependent differences in the permeation of intercellular channels.[22–24] One striking example of the possible importance to hepatocytes is that homomeric connexons made of Cx32 are permeable to both cAMP and cGMP, whereas heteromeric connexons composed of Cx32 and Cx26 lose permeability to cAMP but not to cGMP.[25]

Characterization of the genomic sequence corresponding to Cx32 defined a gene structure[26] that is now appreciated as being common to almost all other members of the connexin gene family. The Cx32 coding sequence is included within one open reading frame on a single exon, which is separated from a tiny upstream exon (~100 bp) by about 6 kb of an intervening intronic sequence. Promoter mapping in human liver-derived cell line HUH-7 localized a minimal basal promoter within a 70-bp region immediately upstream of the mRNA start sites and DNAase hypersensitive sites 1.2 kb downstream of the Cx32 open reading frame.[27,28] More recent experiments have identified positive and negative regulatory domains of the Cx32 promoter and nuclear proteins (hepatocyte nuclear factor, HNF-1) that bind to them from experiments performed on MH1C1 rat

hepatoma cells and WB-F344 rat liver epithelial cells.[29,30] Cx32 expression was significantly decreased in the HNF-1α-deficient mouse liver.[30]

Cx26 has the shortest carboxyl tail of all the connexins thus far sequenced and is not phosphorylated.[21] On the basis of experiments performed in cellfree systems, it has been proposed that Cx26 can insert into membranes post-translationally, whereas Cx32 is believed to insert cotranslationally.[31] Rapid specific appearance of Cx26 in the plasma membrane after perfusion of the female rat liver without an increase in total protein or mRNA levels was reported.[32] Furthermore, brefeldin A, a drug that disrupts the Golgi apparatus, had minimal effects on trafficking of Cx26 to the plasma membrane, in contrast to its disruption of Cx32 trafficking.[33] These findings suggest that the formation of Cx26 homotypic channels on the membranes may follow an alternative nonclassical trafficking pathway bypassing the Golgi system, in contrast to the classical trafficking pathway (ER–IC–Golgi–plasma membrane) followed by Cx32. Cx26 has been proposed to be a tumor suppressor gene, on the basis of subtractive hybridization using normal and malignant human mammary epithelial cells.[34] Cx26 expression in human mammary epithelial cells induced by TPA treatment is controlled at the level of transcriptional modification.[35] Kiang et al. have cloned and sequenced the 5′-portion of the human Cx26 gene, revealing that the promoter region contains six GC boxes, two GT boxes, a TTAAAA box, and a TPA-induced DNase I hypersensitivity region,[36,37] providing a site by which TPA may exert transcriptional control over Cx26. Nevertheless, induction of exogenous Cx26 in neuroblastoma cells by TPA treatment appears to be controlled by posttranslational mechanisms.[38]

Hepatic tight junctions

Tight junctions have a complex molecular composition that has only recently begun to be clarified in detail.[39] The integral membrane components of tight junctions include occludin, the claudin family, and JAM.

The claudin family at present consists of 20 members and two or more distinct claudins are generally coexpressed in single cells of various tissues;[39] for example, claudin-1, -2, and -3 are expressed in the bile canaliculus region of mature hepatocytes (Fig. 2D), while claudin-14 mRNA was detected in the liver.[40] In the rat liver, claudin-2 shows a lobular gradient increasing from periportal to pericentral hepatocytes, whereas claudin 3 is uniformly expressed.[41] Recent detailed analyses of the manner of interaction of heterogeneous claudin species within and between tight-junction strands suggest that distinct species of claudins are copolymerized linearly to form tight-junction strands as homopolymers or heteropolymers and that the claudins interact between each of the paired strands in a homophilic or heterophilic manner, including both other claudins and/or occludin.[42] By contrast, only a single occludin transcript has been described, although an alternatively spliced form of occludin (termed occludin 1B) was reported recently.[43] As with the connexins, the major tight-junction components occludin and the claudins are tetraspan proteins with intracellular amino and carboxyl termini. Several cytoplasmic proteins, including ZO-1, ZO-2, ZO-3, 7H6 antigen, cingulin, symplekin, Rab3B, Ras target AF-6, and ASIP, an atypical protein kinase C interacting protein, have been reported to be associated with tight junctions, and some of these are believed to play roles in signal transduction.[39] The cytoplasmic domains of occludin and claudins are reported to bind to ZO-1, ZO-2, and ZO-3, forming a macromolecular complex at cell membranes.[39] ZO-1, ZO-2, and ZO-3 are members of the membrane-associated guanylate kinase (MAGUK) family of proteins displaying a characteristic multidomain structure composed of SH3, guanylate kinase-like (GUK), and multiple PDZ (PSD95-Dlg-ZO1) domains.[39] Although direct involvement of tight junction-associated proteins in the regulation of gene expression has not been still demonstrated, researchers have recently investigated this possibility.[44–46]

Interaction between gap junctions and tight junctions linked with actin filaments in hepatocytes

Other proteins have been reported to interact with connexis (Table 1). Recently, it has become established that Cx43 interacts with ZO-1 in Cx43 transfectants, in normal fibroblasts, and in cardiac myocytes.[47,48] This interaction is direct, through binding of the extreme carboxyl terminus of Cx43 and the second PDZ domain of ZO-1.[47,48] Furthermore, it is well known that small gap-junction plaques are associated with tight-junction strands in some cell types, including hepatocytes (Fig. 3C). In primary cultured rat hepatocytes, Cx32 is partly colocalized with occludin and claudin-1, in which form tight-junction structures (Fig. 3A). To examine the roles of gap junctions in regulating expression and structure of tight junctions, we transfected human Cx32 cDNA into two immortalized mouse hepatocyte lines (CHST8 cells, which lack endogenous Cx32 and Cx26, and Cx32KOH, derived from Cx32-deficient mouse hepatocytes).[49,50] In Cx32 transfectants, induction of tight-junction strands and the integral tight-junction proteins occludin and claudins were observed, and small gap-junction plaques appeared within the induced tight junction strands. The induced endogenous occludin protein in the transfectants was found to bind to the exogenously expressed Cx32 protein. Furthermore, tight junctional functions, fence, and barrier functions, were increased in Cx32 transfectants.

These results indicate that gap-junction and tight-junction expression are closely correlated in hepatocytes,

Table 1. Other proteins that have been reported to interact with connexins

v-Src and c-Src; Cx43[76–78]
Tubulin; Cx43[79]
Caveolin-1; Cx43[80]
Zona occludens protein-1 (ZO-1); Cx43, Cx45, Cx31.9[47,48,81–83]
Occludin; Cx26, Cx32[49,50,63,84]

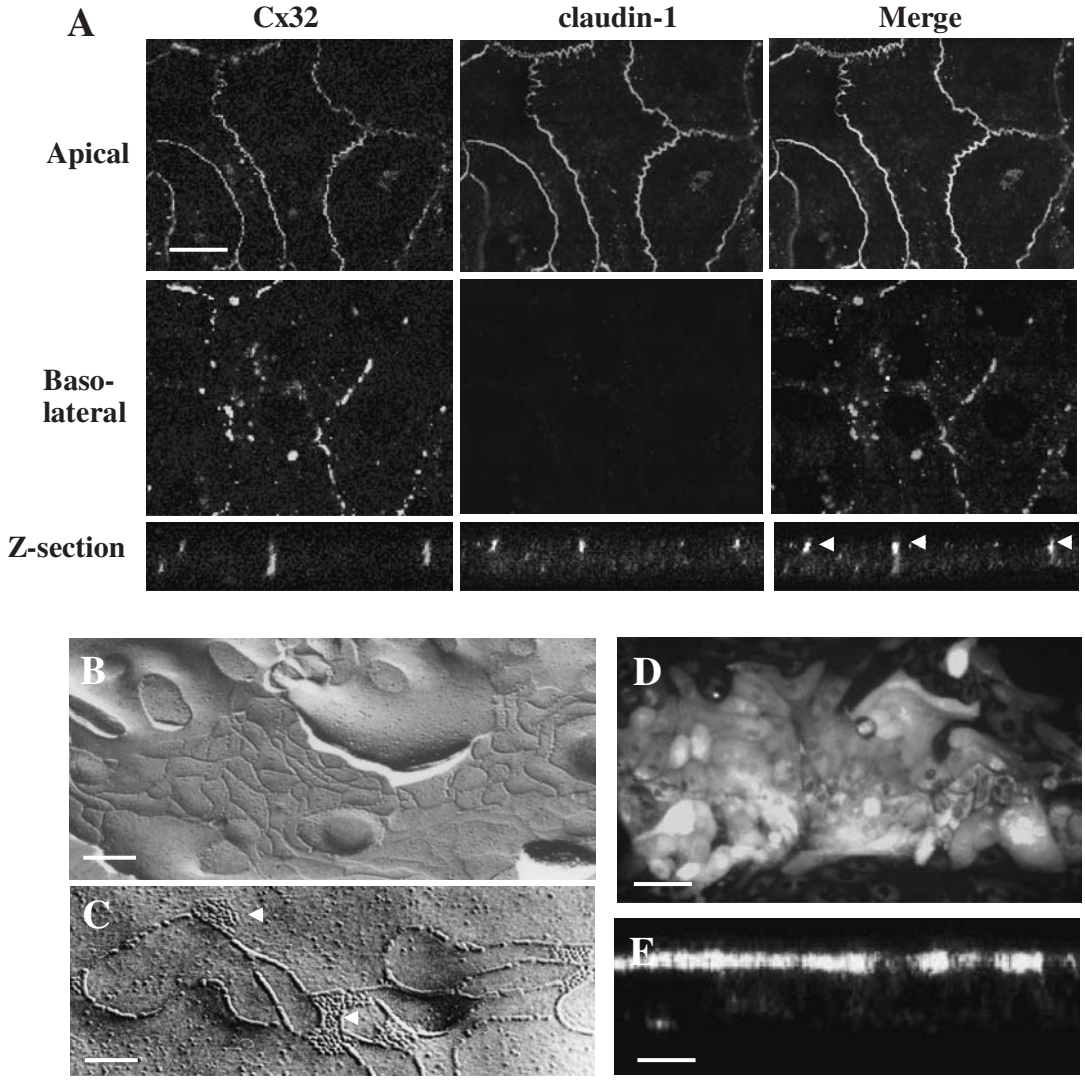

Fig. 3. Interaction between gap junctions and tight junctions in primary cultured rat hepatocytes. **A** Double staining of Cx32 and claudin-1. Cx32-immunoreactive lines are observed on the most subapical plasma membrane at cell borders whereas Cx32-positive spots can be observed on the basolateral membrane. Claudin-1-immunoreactive lines are observed on the most subapical plasma membrane of the cell borders and are colocalized with Cx32-immunoreactive lines (*arrows* in **A**). **B,C** Freeze-fracture. In freeze-fracture analysis of the adluminal plasma membrane, tight-junction strands form well-developed networks (**B**), and many small gap-junction plaques are observed peripherally or within the tight junction network (*arrowheads* in **C**). **D** Gap junctional intracellular communication (GJIC) using scrape-loading/dye transfer by lucifer yellow. **E** Fence function images of tight junctions. The hepatocytes are labeled with BODIPY-sphingomyelin. The fluorescent probe is effectively retained in the apical domain (**D**). *Bars* **A,E** 10 μm; **B** 200 nm; **C** 50 nm; **D** 80 mm

and we speculate that through this association gap-junction expression may play a crucial role in the establishment of cell polarity via regulation of tight-junction proteins. This finding supports previous studies in which gap junctions have been associated with other intercellular junctional components. More importantly, however, the present demonstration of a direct linkage between occludin and Cx32 and previous studies showing high-affinity interactions between ZO-1 and Cx43[47,48] indicate that connexins may form selective associations with specific components of adherens or tight junctions. We have proposed that, through binding of these proteins to cytoskeletal, adhesion, and signaling proteins, different connexins may promote the aggregation of connexin-specific scaffolds at junctional regions, providing not only intercellular signaling but also sites where intracellular signaling is transduced.[51] Interestingly, in the liver in HNF-1α-deficient mice, not only a decrease of Cx32 expression but also lack of expression of claudin-2 mRNA and protein was observed, whereas claudin-1 mRNA expression was unaffected.[45]

Using electron microscopy, actin filaments have been localized near the cytoplasmic side of tight junctions, and some integral or peripheral tight-junction proteins that bind to actin filaments have been seen.[39,52,53] ZO-1 and ZO-2 also bind to actin filaments,[53] suggesting a role of tight junctions in the organization of the actin cytoskeleton. Gap junctions are also associated with actin filaments. More recently, forced expression of connexins was found to be accompa-

nied by a profound reorganization of the actin and myosin cytoskeleton in Cx32-tranfected C6 glioma cells.[54] Gap junctions assemble in the presence of cytoskeletal inhibitors, but enhanced assembly requires microtubules.[55] Primary cultured hepatocytes from Cx32-deficient mice showed the intensity of actin stress fibers.[56] In the Cx32 transfectants in which the human Cx32 gene was transfected to a mouse hepatocytic cell line derived from Cx32-deficient primary mouse hepatocytes, circumferential actin filaments were increased without a change of actin protein.[50] These results suggest that a novel linkage may exist between gap junctions and cellular morphology via organization of the actin cytoskeleton in hepatocytes.

Gap and tight junctions in primary cultures of rat hepatocytes

Cx32 reexpression in primary cultured rat hepatocytes is dramatically enhanced in medium containing epidermal growth factor (EGF) and 2% dimethylsulfoxide (DMSO),[57] and Cx26 can also be induced when glucagon is added together with 2% DMSO.[58,59] Primary rat hepatocytes cultured with DMSO/glucagon have extensive gap junctional intercellular communication (GJIC), as measured using scrape-loading/dye transfer by lucifer yellow (Fig. 3D), and Cx32- but not Cx26-immunoreactive lines are observed on the most subapical plasma membrane at cell borders (Fig. 3A), while on the basolateral membrane both Cx32- and Cx26-positive spots are colocalized. These latter studies suggest that expression of gap junctions in hepatocytes may be closely related to oxidative stress and that oxygen radical scavengers such as DMSO or melatonin might be important inducing substances.[60,61] During stimulation and reinhibition of DNA synthesis, differential changes of expression and function of Cx32 and Cx26 were observed.[62]

In freeze-fracture analysis of primary rat hepatocytes cultured with DMSO/glucagon, tight-junction strands formed well-developed networks, and many gap-junction plaques were observed within the strand network (Fig. 3B,C), Occludin, claudin-1-, ZO-1-, and ZO-2-immunoreactive lines were strongly observed on the most subapical plasma membrane of the cell borders and were colocalized with Cx32-immunoreactive lines (Fig. 3A). Cx32 protein in hepatocytes may bind to tight-junction proteins, occludin, claudin-1, and ZO-1.[63] The fence function of tight junctions in the cells, as examined by diffusion of labeled sphingomyelin, was well maintained (Fig. 3E). In a more recent study, treatment with GJIC blockers, 18β GA and oleamide, inhibited expression of occludin mRNA and protein in hepatocytes. Furthermore, changes of tight-junction strands and occludin expression are observed during DNA synthesis in cultured hepatocytes.[62,64] Because tight junctions in these differentiated cultured hepatocytes assume the distribution seen in simple polarized epithelial cells, this culture system may provide a useful model in which to study hepatocyte tight junctions.

In primary rat hepatocytes cultured with DMSO/glucagon, circumferential actin filaments were also observed near the tight-junction regions.[59,65] Treatment of the cells with the actin depolymerizing drug mycalolide B caused the disappearance of both the circumferential actin filaments and occludin, whereas tight-junction strands remained virtually intact, leading to the hypothesis that occludin, but not other transmembrane proteins, plays a role in the linkage between the actin cytoskeleton and tight junctions in hepatocytes.[66]

Perspectives

The signal transduction pathways, activation of mitogen-activated protein (MAP)-kinase and the phosphoinositol 3-kinase (PI3-kinase) signaling cascade, regulate a variety of cellular processes including proliferation, differentiation, and transformation.[67] A role of gap and tight junctions in inter- or intracellular signaling has been proposed. To elucidate the mechanisms of signal transmission required for the regulation of the gap and tight junctions, researchers have recently examined the effects of signaling pathways such as MAP kinase and PI3 kinase on the regulation of gap and tight junctions.[68–74] Our recent experiments indicated that treatment with growth factors [EGF and transforming growth factor-beta (TGF-β)] or proinflammatory cytokine (IL-1β) may induce downregulation of Cx32 and claudin-1 or upregulation of claudin-2 in primary rat hepatocytes via signaling pathways such as MAP kinase, p38 MAP kinase, and PI3 kinase. It is possible that loss of gap junctions and leaky tight junctions in cholestatic hepatocytes may be caused by various growth factors and proinflammatory cytokines through the signaling pathways.

Although gap and tight junctions perform very different functions, there are numerous points at which functions overlap. Indeed, the findings that the traditionally tight junction-associated protein ZO-1 also binds to a connexin, and that occludin is colocalized with Cx32 in transfectants, indicate the possibility for either coordinate or reciprocal regulation of macromolecular complexes containing gap- and tight-junction proteins. Studies of protein–protein interactions and of coordinate and subordinate regulation of gene families are expected to soon disclose the intricacies of inter- and intracellular signaling and growth control at gap junctions and the regulatory mechanisms of the blood–biliary barrier formed by tight junctions.[75]

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References

1. Traub O, Druge PM, Willecke K (1983) Degradation and resynthesis of gap junction protein in plasma membranes of regenerating

liver after partial hepatectomy or cholestasis. Proc Natl Acad Sci USA 80:755–759
2. Fallon MB, Nathanson MH, Mennone A, Saez JC, Burgstahler AD, Anderson JM (1995) Altered expression and function of hepatocyte gap junctions after common bile duct ligation in the rat. Am J Physiol 268:C1186–C1194
3. Anderson JM (1996) Leaky junctions and cholestasis: a tight correlation. Gastroenterology 110:1662–1665
4. Trauner M, Meier PJ, Boyer JL (1998) Molecular pathogenesis of cholestasis. N Engl J Med 339:1217–1227
5. Takakuwa Y, Kokai Y, Sasaki K, Chiba H, Tobioka H, Mori M, Sawada N (2002) Bile canalicular barrier function and expression of tight-junctional molecules in rat hepatocytes during common bile duct ligation. Cell Tissue Res 307:181–189
6. Phillips MJ, Poucell S, Oda M (1986) Mechanisms of cholestasis. Lab Invest 54:593–608
7. Song JY, Van Noorden CJ, Frederiks WM (1998) Rearrangement of hepatocellular F-actin precedes the formation of rosette-like structures in parenchyma of cholestatic rat liver. Hepatology 27:765–771
8. Watanabe S, Phillips MJ (1984) Ca^{2+} causes active contraction of the bile cannaliculi: direct evidence from microinjection studies. Proc Natl Acad Sci USA 81:6164–6165
9. Nathanson MH, Rios-Velez L, Burgstahler AD, Mennone A (1999) Communication via gap junctions modulates bile secretion in the isolated perfused rat liver. Gastroenterology 116:1176–1183
10. Yee AG, Revel JP (1978) Loss and reappearance of gap junctions in regenerating liver. J Cell Biol 78:554–564
11. Yancey SB, Easter E, Revel JP (1979) Cytological changes in gap junctions during liver regeneration. J Ultrastruct Res 67:229–242
12. Easter DW, Wade JB, Boyer JL (1983) Structural integrity of hepatocyte tight junctions. J Cell Biol 96:745–749
13. Tsukita S, Furuse M (1999) Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell Biol 9:268–273
14. Graf J, Gautam A, Boyer JL (1984) Isolated rat hepatocyte couplets: a primary secretory unit for electrophysiologic studies of bile secretory function. Proc Natl Acad Sci USA 81:6516–6520
15. Claude P, Goodenough DA (1973) Fracture faces of zonulae occludentes from "tight" and "leaky" epithelia. J Cell Biol 58:390–400
16. Barth CA, Schwartz LR (1982) Transcellular transport of fluorescein in hepatocyte monolayers: evidence for functional polarity of cells in culture. Proc Natl Acad Sci USA 79:4985–4987
17. Kumar NM, Gilula NB (1996) The gap junction communication channel. Cell 84:381–388
18. Shibata Y, Kumai M, Nishi K, Nakamura K (2001) Diversity and molecular anatomy of gap junctions. Med Electron Microsc 34:153–159
19. Phelan P, Bacon JP, Davies JA, Stebbings LA, Todman MG, Avery L, Baines RA, Barnes TM, Ford C, Hekimi S, Lee R, Shaw JE, Starich TA, Curtin KD, Sun YA, Wyman RJ (1998) Innexins: a family of invertebrate gap-junction proteins. Trends Genet 14:348–349
20. Stauffer KA (1995) The gap junction proteins beta 1-connexin (connexin-32) and beta 2-connexin (connexin-26) can form heteromeric hemichannels. J Biol Chem 270:6768–6772
21. Valiunas V, Niessen H, Willecke K, Weingart R (1999) Electrophysiological properties of gap junction channels in hepatocytes isolated from connexin32-deficient and wild-type mice. Pfleugers Arch 437:846–856
22. Elfgang C, Eckert R, Lichtenberg-Fraté H, Butterweck A, Traub O, Klein RA, Hülser DF, Willcke K (1995) Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. J Cell Biol 129:805–817
23. Cao F, Eckert R, Elfgang C, Nitsche JM, Snyder S, Hülser DF, Willcke K, Nicholson BF (1998) A quantitative analysis of connexin-specific permeability differences of gap junctions expressed in HeLa transfectants and *Xenopus* oocytes. J Cell Sci 111:31–43
24. Niessen H, Willcke K (2000) Strongly decreased gap junctional permeability to inositol 1,4,5-trisphosphate in connexin32 deficient hepatocytes. FEBS Lett 146:112–114
25. Bevans CG, Kordel M, Rhee SK, Harris AL (1998) Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules. J Biol Chem 273:2808–2816
26. Miller T, Dahl G, Werner R (1988) Structure of gap junction gene: rat connexin32. Biosci Rep 8:455–461
27. Bai S, Spray DC, Burk RD (1993) Identification of proximal and distal regulatory elements of the connexin32 gene. Biochim Biophys Acta 1216:197–204
28. Bai S, Schoenfeld A, Píetrangelo A, Burk RD (1995) Basal promoter of the rat connexin 32 gene: identification and characterization of an essential element and its DNA-binding protein. Mol Cell Biol 15:1439–1445
29. Piechocki MP, Toti RM, Fernstrom MJ, Burk RD, Ruch RJ (2000) Liver cell-specific transcriptional regulation of connexin32. Biochim Biophys Acta 1491:107–122
30. Koffler LD, Fernstrom MJ, Akiyama TE, Gonzalez FJ, Ruch RJ (2002) Positive regulation of connexin32 transcription by hepatocyte nuclear factor-1-alpha. Arch Biochem Biophys 407:160–167
31. Zhang JT, Chen M, Foote CI, Nicholson BJ (1996) Membrane integration of in vitro-translated gap junctional proteins: co-and post-translational mechanisms. Mol Biol Cell 7:471–482
32. Kojima T, Sawada N, Oyamada M, Chiba H, Isomura H, Mori M (1994) Rapid appearance of connexin 26-positive gap junctions in centrilobular hepatocytes without induction of mRNA and protein synthesis in isolated perfused liver of female rat. J Cell Sci 107:3579–3590
33. Evans WH, Ahmad S, Diez J, George CH, Kendall JM, Martin PE (1999) Trafficking pathways leading to the formation of gap junctions. Novartis Found Symp 219:44–54
34. Lee SW, Tomasetto C, Paul D, Keyomarsi K, Sager R (1992) Transcriptional downregulation of gap junction proteins blocks junctional communication in human mammary tumor cell line. J Cell Biol 118:1213–1221
35. Li GY, Lin HH, Tu ZJ, Kiang DT (1998) Gap junction Cx26 gene modulation by phorbol esters in benign and malignant human mammary cells. Gene (Amst) 209:139–147
36. Kiang DT, Tu ZJ, Lin HH (1997) Upstream genomic sequence of the human connexin 26 gene. Gene (Amst) 199:165–171
37. Tu ZJ, Kiang DT (1998) Mapping and characterization of the basal promoter of human connexin 26 gene. Biochim Biophys Acta 1443:169–181
38. Kojima T, Srinivas M, Fort A, Hopperstad M, Urban M, Hertzberg EL, Mochizuki Y, Spray DC (1999) TPA induced expression and function of human connexin 26 by post-translational mechanisms in stably transfected neuroblastoma cells. Cell Struct Funct 24:435–441
39. Tsukita S, Furuse M, Itoh M (2001) Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2:285–293
40. Wilcox ER, Burton QL, Naz S, Riazuddin S, Smith TN, Ploplis B, Belyantseva I, Ben-Yosef T, Liburd NA, Morell RJ, Kachar B, Wu DK, Griffith AJ, Riazuddin S, Friedman TB (2001) Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. Cell 104:165–172
41. Rahner C, Mitic LL, Anderson JM (2001) Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut. Gastroenterology 120:411–422
42. Furuse M, Sasaki H, Tsukita S (1999) Manner of interaction of heterogenous claudin species within and between tight junction strands. J Cell Biol 147:891–903
43. Muresan Z, Paul DL, Goodenough DA (2000) Occludin1B, a variant of the tight junction protein occludin. Mol Biol Cell 11:627–634
44. Balda MS, Matter K (2000) The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. EMBO J 19:2024–2033
45. Sakaguchi T, Gu X, Golden HM, Suh E, Rhoads DB, Reinecker HC (2002) Cloning of the human claudin-2 5′-flanking region revealed a TATA-less promoter with conserved binding sites in mouse and human for caudal-related homeodomain proteins and hepatocyte nuclear factor-1alpha. J Biol Chem 277:21361–21370
46. Mankertz J, Waller JS, Hillenbrand B, Tavalali S, Florian P, Schoneberg T, Fromm M, Schulzke JD (2002) Gene expression of the tight junction protein occludin includes differential splicing and alternative promoter usage. Biochem Biophys Res Commun 298:657–666
47. Giepmans BNG, Moolenaar WH (1998) The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. Curr Biol 8:931–934

48. Toyofuku T, Yabuki M, Otsu K, Kuzuya T, Hori M, Tada M (1998) Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. J Biol Chem 273:12725–12731
49. Kojima T, Sawada N, Chiba H, Kokai Y, Yamamoto M, Urban M, Lee G-H, Hertzberg EL, Mochizuki Y, Spray DC (1999) Induction of tight junctions in human connexin 32 (hCx32)-transfected mouse hepatocytes: connexin 32 interacts with occludin. Biochem Biophys Res Commun 266:222–229
50. Kojima T, Spray DC, Kokai Y, Chiba H, Mochizuki Y, Sawada N (2002) Cx32 formation and/or Cx32-mediated intercellular communication induces expression and function of tight junctions in hepatocytic cell line. Exp Cell Res 276:40–51
51. Spray DC, Duffy HS, Scemes E (1999) Gap junctions in glia: types, roles and plasticity. In: The function of glial cells in health and disease: dialogue between glia and neurons. Adv Exp Med Biol 468:339–359
52. Fanning AS, Mitic LL, Anderson JM (1999) Transmembrane proteins in the tight junction barrier. J Am Soc Nephrol 10:1337–1345
53. Wittchen ES, Haskins J, Stevenson BR (1999) Protein interactions at the tight junction. Actin has multiple binding partners, and ZO-1 forms independent complexes with ZO-2 and ZO-3. J Biol Chem 274:35179–35185
54. Cotrina ML, Lin JH, Lopez-Garcia JC, Naus CC, Nedergaard M (2000) ATP-mediated glia signaling. J Neurosci 20:2835–2844
55. Johnson RG, Meyer RA, Li XR, Preus DM, Tan L, Grunenwald H, Paulson AF, Laird DW, Sheridan JD (2002) Gap junctions assemble in the presence of cytoskeletal inhibitors, but enhanced assembly requires microtubules. Exp Cell Res 275:67–80
56. Kojima T, Fort A, Tao M, Yamamoto M, Spray DC (2001) Gap junction expression and cell proliferation in differentiating cultures of Cx32 KO mouse hepatocytes. Am J Physiol Gastrointest Liver Physiol 281:G1004–G1013
57. Kojima T, Mitaka T, Paul D, Mori M, Mochizuki Y (1995) Reappearance and long-term maintenance of connexin 32 in proliferated adult rat hepatocytes: use of serum-free L-15 medium supplemented with EGF and DMSO. J Cell Sci 108:1347–1357
58. Kojima T, Mitaka T, Shibata Y, Mochizuki Y (1995) Induction and regulation of connexin 26 by glucagon in primary cultures of adult rat hepatocytes. J Cell Sci 108:2771–2780
59. Kojima T, Yamamoto M, Tobioka H, Mizuguchi T, Mitaka T, Mochizuki Y (1996) Changes in cellular distribution of connexin 32 and 26 during formation of gap junctions in primary cultures of adult rat hepatocytes. Exp Cell Res 223:314–326
60. Kojima T, Mitaka T, Mizuguchi T, Mochizuki Y (1996) Effects of oxidant radical scavengers on connexins 32 and 26 expression in primary cultures of adult rat hepatocytes. Carcinogenesis (Oxf) 17:537–544
61. Kojima T, Mochizuki C, Mitaka T, Mochizuki Y (1997) Effects of melatonin on proliferation, oxidative stress and Cx32 gap junction protein expression in primary cultures of adult rat hepatocytes. Cell Struct Funct 22:347–356
62. Kojima T, Yamamoto M, Mochizuki C, Mitaka T, Sawada N, Mochizuki Y (1997) Different changes in expression and function of connexin 26 and connexin 32 during DNA synthesis and redifferentiation in primary rat hepatocytes using a DMSO culture system. Hepatology 26:585–597
63. Kojima T, Kokai Y, Chiba H, Yamamoto M, Mochizuki Y, Sawada N (2001) Cx32 but not Cx26 is associated with tight junctions in primary cultures of rat hepatocytes. Exp Cell Res 263:193–201
64. Kojima T, Sawada N, Kokai Y, Yamamoto M, Mori M, Mochizuki Y (1998) Occludin expression and tight junction strand formation during replicative DNA synthesis in primary cultures of rat hepatocytes. Med Electron Microsc 31:169–176
65. Kojima T, Mochizuki C, Tobioka H, Saitoh M, Takahashi S, Mitaka T, Mochizuki Y (1997) Formation of actin filament networks in cultured rat hepatocytes treated with DMSO and glucagon. Cell Struct Funct 22:269–278
66. Kojima T, Sawada N, Yamamoto M, Kokai Y, Mori M, Mochizuki Y (1999) Disruption of circumferential actin filament causes disappearance of occludin from the cell borders of rat hepatocytes in primary culture without distinct changes of tight junctions. Cell Struct Funct 24:11–17
67. Cobb MH, Hepler JE, Cheng M, Robbins D (1994) The mitogen-activated protein kinases, ERK1 and ERK2. Semin Cancer Biol 5:261–268
68. Kanemitsu MY, Lau AF (1993) Epidermal growth factor stimulates the disruption of gap junctional communication and connexin43 phosphorylation independent of 12-*O*-tetradecanoylphorbol 13-acetate-sensitive protein kinase C: the possible involvement of mitogen-activated protein kinase. Mol Biol Cell 4:837–848
69. Yao J, Morioka T, Oite T (2000) PDGF regulates gap junction communication and connexin43 phosphorylation by PI 3-kinase in mesangial cells. Kidney Int 57:1915–1926
70. Potempa S, Ridley AJ (1998) Activation of both MAP kinase and phosphatidylinositide 3-kinase by *ras* is required for hepatocyte growth factor/scatter-induced adherens junction disassembly. Mol Biol Cell 9:2185–21200
71. Woo PL, Ching D, Guan Yi, Firestone GL (1999) Requirement for *ras* and phosphatidylinositide 3-kinase signaling uncouples the glucocorticoid-induced junctional organization and transepithelial electrical resistance in mammary tumor cells. J Biol Chem 274:32818–32828
72. Li D, Mrsny RJ (2000) Oncogenic raf-1 disrupts epithelial tight junctions via downregulation of occludin. J Cell Biol 148:791–800
73. Chen Yh, Lu Q, Schneeberger EE, Goodenough DA (2000) Restoration of tight junction structure and barrier function by down-regulation of the mitogen-activated protein kinase pathway in ras-transformed Madin-Darby canine kidney cells. Mol Biol Cell 11:849–862
74. Kinugasa T, Sakaguchi T, Gu X, Reinecker HC (2000) Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology 118:1001–1011
75. Kojima T, Sawada N, Duffy HS, Spary DC (2001) Gap and tight junctions in liver: composition, regulation and function. In: Arias IM, Boyer JL, Chisari FV, Fausto N, Schachter D, Shafritz DA (eds) The liver-biology and pathobiology, 4th edn. Lippincott Williams & Wilkins, Philadelphia, pp 29–46
76. Loo LW, Kanemitsu MY, Lau AF (1999) In vivo association of pp60v-src and the gap-junction protein connexin 43 in v-src-transformed fibroblasts. Mol Carcinog 25:187–195
77. Toyofuku T, Akamatsu Y, Zhang H, Kuzuya T, Tada M, Hori M (2001) c-Src regulates the interaction between connexin-43 and ZO-1 in cardiac myocytes. J Biol Chem 276:1780–1788
78. Giepmans BN, Hengeveld T, Postma FR, Moolenaar WH (2001) Interaction of c-Src with gap junction protein connexin-43. Role in the regulation of cell-cell communication. J Biol Chem 276:8544–8549
79. Giepmans BN, Verlaan I, Hengeveld T, Janssen H, Calafat J, Falk MM, Moolenaar WH (2001) Gap junction protein connexin-43 interacts directly with microtubules. Curr Biol 11:1364–1368
80. Schubert AL, Schubert W, Spray DC, Lisanti MP (2002) Connexin family members target to lipid raft domains and interact with caveolin-1. Biochemistry 41:5754–5764
81. Kausalya PJ, Reichert M, Hunziker W (2001) Connexin45 directly binds to ZO-1 and localizes to the tight junction region in epithelial MDCK cells. FEBS Lett 505:92–96
82. Laing JG, Manley-Markowski RN, Koval M, Civitelli R, Steinberg TH (2001) Connexin45 interacts with zonula occludens-1 and connexin43 in osteoblastic cells. J Biol Chem 276:23051–23055
83. Nielsen PA, Beahm DL, Giepmans BN, Baruch A, Hall JE, Kumar NM (2002) Molecular cloning, functional expression, and tissue distribution of a novel human gap junction-forming protein, connexin-31.9. Interaction with zona occludens protein-1. J Biol Chem 277:38272–38283
84. Nusrat A, Chen JA, Foley CS, Liang TW, Tom J, Cromwell M, Quan C, Mrsny RJ (2000) The coiled-coil domain of occludin can act to organize structural and functional elements of the epithelial tight junction. J Biol Chem 275:29816–29822