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MULTI-AUTHOR REVIEW

Roles of connexins and pannexins in digestive homeostasis

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Abstract Connexin proteins are abundantly present in the digestive system. They primarily form gap junctions, which control the intercellular exchange of critical homeostasis regulators. By doing so, gap junctions drive a plethora of gastrointestinal and hepatic functional features, including gastric and gut motility, gastric acid secretion, intestinal innate immune defense, xenobiotic biotransformation, glycogenolysis, bile secretion, ammonia detoxification and plasma protein synthesis. In the last decade, it has become clear that connexin hemichannels, which are the structural precursors of gap junctions, also provide a pathway for cellular communication, namely between the cytosol and the extracellular environment. Although merely pathological functions have been described, some physiological roles have been attributed to connexin hemichannels, in particular in the modulation of colonic motility. This equally holds true for cellular channels composed of pannexins, connexin-like proteins recently identified in the intestine and the liver, which have become acknowledged key players in inflammatory processes and that have been proposed to control colonic motility, secretion and blood flow.

Keywords Stomach · Intestine · Liver · Pannexin · Connexin · Physiology

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Abbreviations

ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CL	Cytoplasmic loop
CT	Cytoplasmic carboxy tail
Cx	Connexin
EL	Extracellular loop
GJIC	Gap junctional intercellular communication
IP_3	Inositol triphosphate
NT	Cytoplasmic amino tail
Panx	Pannexin
TM	Transmembrane domain

Introduction

Like in all other organs, homeostasis in the digestive system is dictated by the interplay between intracellular, extracellular and intercellular communication networks. Direct intercellular communication is typically governed by gap junctions, composed of 2 hemichannels of neighboring cells, which control the diffusion of small and hydrophilic chemical substances between adjacent cells. This flux is called gap junctional intercellular communication (GJIC) and involves several second messengers, such as adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP) and inositol triphosphate (IP_3) as well as ions, including calcium and sodium [1–3]. Gap junctions have been first described in 1967 in liver cells [4, 5]. In 1974, Goodenough isolated 2 gap junctional proteins from mouse liver and called them connexin (Cx) proteins [6]. The cloning of these first 2 connexins from rat liver was performed in 1986 [7, 8], of which one was simultaneously detected in rat stomach [7], and was the start of

almost 3 decades of intensive gap junction research. Over the years, as much as 21 different connexins have been identified in human, all which are named after their molecular weight, namely Cx23, Cx25, Cx26, Cx30, Cx30.2, Cx30.3, Cx31, Cx31.1, Cx31.9, Cx32, Cx36, Cx37, Cx40, Cx40.1, Cx43, Cx45, Cx46, Cx47, Cx50, Cx59 and Cx62 [9]. Connexins share a structure consisting of 4 membrane-spanning domains, 2 extracellular loops, a cytoplasmic loop, a cytoplasmic N-terminal area and a C-terminal region [2, 3] (Fig. 1). Started in 1993 [10, 11], several studies have documented the presence of connexins in the intestine. In 2000, a newcomer entered the connexin arena, namely the so-called pannexin (Panx) protein, which has a topology that is similar to that of connexins [12]. Thus far, 3 pannexin types, namely Panx1, Panx2 and Panx3, have been characterized and they gather in a connexin hemichannel-like configuration, but not as gap junctions, at the cell plasma membrane surface, where they mediate the exchange of chemical messengers between the cytosol and the extracellular environment [13] (Fig. 1). Only in the last few years, however, pannexin expression has been reported in the intestine [14, 15], the stomach [15, 16] and the liver [15–22]. Furthermore, the connexin research field has witnessed the introduction of the controversial concept of functional hemichannels in the last decade. In this view, connexin hemichannels are more than merely structural precursors of gap junctions, as they also provide a pathway for cellular signaling, albeit between the cytosol and the extracellular environment, similar to pannexin channels [23, 24]. The messengers that permeate connexin hemichannels and pannexin channels show great overlap with those involved in GJIC [13, 25]. Over the years, several studies have documented that these single membrane channels are mainly involved in intestinal [14, 26–28] and liver pathology [29, 30]. Nevertheless, a number of physiological roles of connexin hemichannels and pannexin channels have been described in the digestive system, all which will be outlined in the current paper. In the first part, a state-of-the-art overview of connexin and pannexin expression in the stomach, the intestine and the liver is provided. The second part focuses on physiological functions of connexin and pannexin signaling in these organs.

Connexins and pannexins in the stomach, the intestine and the liver

Gastric connexins and pannexins

Today, 3 connexin isotypes have been identified in the stomach, namely Cx26, Cx32 and Cx43 (Table 1). In particular, Cx26 has been detected in human [31], mouse [32] and rat [33, 34] gastric tissue, where it is only scarcely

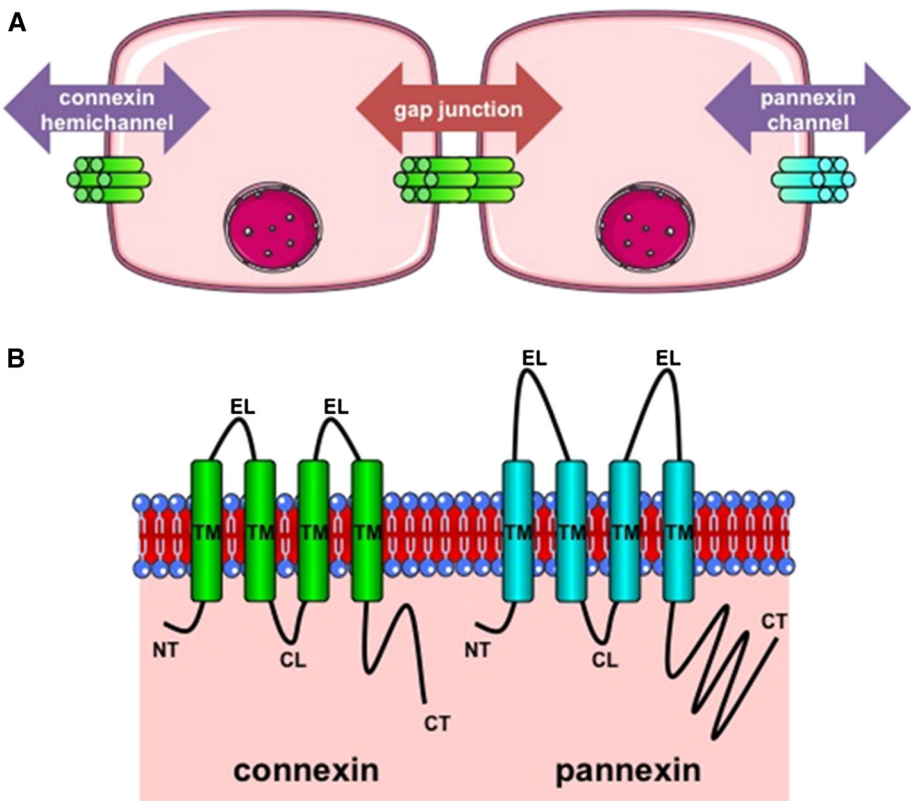

Fig. 1 **a** Architecture of connexin and pannexin channels. Gap junctions are formed by the interaction between 2 hemichannels of adjacent cells and mediate intercellular communication (*red arrow*). Connexin hemichannels and pannexin channels are built up by 6 connexin proteins (*green*) and 6 pannexin proteins (*blue*), respectively, and support paracrine communication (*purple*). **b** Topology of connexin and pannexin proteins. Connexins (*green*) and pannexins (*blue*) all consist of 4 transmembrane domains (TM), 2 extracellular loops (EL), 1 cytoplasmic loop (CL), 1 cytoplasmic carboxy tail (CT) and cytoplasmic amino tail (NT). In comparison with connexins, pannexins have longer EL and CT areas

Table 1 Connexin and pannexin expression in the digestive system

Species	Tissue	Cell type	References
Cx26	Stomach (h, m, r)		[31–34]
	Small intestine (h)		[59]
	Colon (h)	Epithelial cells (h)	[59, 74, 78, 151]
		Muscularis externa cells (h)	[75, 151]
	Liver (h, m, r, gp)	Hepatocytes (m, r)	[33, 152, 153]
		Stellate cells (r)	[83]
		Sinusoidal endothelial cells (r)	[83]
		Kupffer cells (r)	[83]
Cx31	Small intestine (m)		[64]
	Colon (m)		[64]
Cx31.9	Colon (h)		[78]
Cx32	Stomach (h, hs, r)	Foveolar cells (h, hs)	[34, 35, 37–43]
	Small intestine (h, m)	Epithelial cells (h, m)	[59, 61, 64]
	Colon (h)	Epithelial cells (h)	[70, 78, 151]
	Liver (h, m, r, gp)	Hepatocytes (h, m, r)	[7, 8, 83, 92, 152–155]
		Biliary endothelial cells (r)	[123]
		Sinusoidal endothelial cells (r)	[83]
Cx36	Small intestine (m)	Myenteric plexus cells (m)	[60]
	Colon (h, m)	Myenteric plexus cells (m)	[60, 78]
Cx37	Small intestine (h, m)	Epithelial cells (h)	[58, 68]
	Liver (m, r)	Hepatic artery endothelial cells (m, r)	[85–87]
		Portal vein endothelial cells (m, r)	[85–87]
Cx40	Stomach (d)		[49]
	Small intestine (d)	Deep muscular plexus cells (d)	[49]
		Myenteric plexus cells (d)	[49]
		Muscularis externa cells (d)	[49]
	Colon (d, r)	Myenteric plexus cells (d)	[49]
		Muscularis externa cells (r)	[71]
	Liver (m, r)	Hepatic artery endothelial cells (m, r)	[85–87]
		Portal vein endothelial cells (m, r)	[85–87]
Cx43	Stomach (h, m, d, r, rb, gp)		[32, 44–53, 156]
	Small intestine (h, m, d, r, rm)	Epithelial cells (h, m, rm)	[59, 61, 62, 64, 69]
		Deep muscular plexus cells (d, r)	[49, 67]
		Myenteric plexus cells (d)	[49]
		Muscularis externa cells (h, m, d, r)	[11, 44, 49, 65]
		Interstitial cells of Cajal (h)	[66]
	Colon (h, m, d, r, rm)	Epithelial cells (h, m)	[59, 64, 69, 70, 72, 73, 77–79, 151]
		Muscularis mucosae cells (h)	[73, 151]
		Deep muscular plexus cells (m, d)	[49, 76]
		Myenteric plexus cells (m, d)	[49, 76]
		Muscularis externa cells (h, r)	[71, 75, 151]
	Liver (h, m, r)	Biliary epithelial cells (r)	[123, 124, 157–161]
		Kupffer cells (r)	[82, 83]
		Stellate cells (r)	[83, 85]
		Sinusoidal endothelial cells (r)	[83, 85]
		Hepatic artery endothelial cells (m, r)	[85–87]
		Portal vein endothelial cells (m, r)	[85–87]

Table 1 continued

Species	Tissue	Cell type	References
Cx45	Stomach (d)		[49]
	Small intestine (d, r)	Deep muscular plexus cells (d, r)	[49, 65, 67]
		Myenteric plexus cells (d)	[49]
	Colon (h, d)	Myenteric plexus cells (d)	[49, 78]
Cx57	Small intestine (m)		[63]
Panx1	Colon (h)	Mucosal cells (h)	[14]
		Muscularis mucosa cells (h)	[14]
		Submucosal cells (h)	[14]
		Muscularis externa cells (h)	[14]
	Liver (m, r)	Hepatocytes (m, r)	[16–20]
		Kupffer cells (m)	[22]
Panx2	Colon (m)	Epithelial cells (m)	[15]
	Liver (m, r)	Hepatocytes (m, r)	[15, 21]

Cx connexin, *d* dog, *gp* guinea pig, *h* human, *hs* horse, *m* mouse, *Panx* pannexin, *r* rat, *rb* rabbit, *rm* rhesus monkey

expressed in the epithelial cells and lamina propria of the fundus [31]. Cx32 is produced in human [35–38], rat [7, 33, 34, 39–42] and equine stomach [43]. In the glandular regions, Cx32 is abundantly expressed in surface and foveolar cells and decreases towards the proliferative zone of the glands, where immature forms of surface epithelial cells are found [35, 40, 41, 43]. Cx43 has been observed in gastric tissue of human [44], mouse [32, 45], rat [46–48], dog [49], rabbit [50] and guinea pig [51–53]. Cx43 is specifically present in circular muscle layers, but not in longitudinal muscle cells, of the antrum and the corpus [44, 45, 49, 51]. In fact, circular muscle cells of the lesser curvature in the corpus of canine stomach are interconnected by numerous gap junctions, whereby each cell has about 200 gap junctions [54]. In human stomach, gap junctions are found in the antrum and in the outermost area of the greater curvature, but are absent in the fundus and in the innermost area of the corporal circular muscle layer [55]. In general, gap junctions are rarely seen at the pylorus [45, 56] and in interstitial cells of Cajal [54], yet in rat, gap junctions are present between intramuscular vagal mechanoreceptors and interstitial cells of Cajal in the fundus [48, 57]. A single study showed expression of Cx40 and Cx45 in circular muscle cells of the canine gastric antrum [49]. Panx1 mRNA expression in the rat gastric tissue has been reported [16], while Panx2 protein expression has been described in parietal and epithelial cells of murine stomach [15] (Table 1).

Intestinal connexins and pannexins

At least 10 different connexin variants have been characterized in the intestinal system. Thus, Cx26, Cx31, Cx32, Cx36, Cx37, Cx40, Cx43, Cx45 and Cx57 have been detected in the small intestine of several species [11, 44, 49, 58–69], while Cx26, Cx31, Cx31.1, Cx32, Cx36, Cx40, Cx43 and Cx45 occur in the colon [49, 59, 60, 64, 70–79] (Table 1). In some reports, the segments of the small intestine in which connexins are produced have been specified. In this regard, Cx31, Cx32 [64], Cx36 [60], Cx43 [49, 62, 64] and Cx45 [49] are present in the ileum, while the duodenum harbors Cx32 [64], Cx37 [58] and Cx43 [64]. Several studies have focussed on the distribution of connexins in the different layers of small intestinal and colonic tissue, in particular the mucosa, submucosa and muscularis externa (Table 1). Furthermore, intestinal connexins, like in other organs, display cell type-specific expression patterns. This might be of importance for delineating physiological compartments with specific functions. Cx32 has been observed in enterochromaffin cells and Paneth cells of small intestinal epithelium in mouse [61]. In rhesus monkeys, Cx43 is exclusively present in crypt epithelial cells, with higher expression in the jejunum and the ileum than in the colon [69]. In the enteric nervous system of the mouse colon, Cx43 is confined to glia [76]. Cx43 is also expressed by circular muscle cells of the small intestine [11, 65], but Cx26, Cx32 and Cx43 immunoreactivity remain absent in the longitudinal muscle layer of the colon [11, 74]. This is linked to the observation that gap junctions occur at specific areas in intestinal tissue. In the mouse intestine, there are no gap junctions between interstitial cells of Cajal of the myenteric plexus and circular or longitudinal muscle cells. With the exception of the latter, however, gap junctions couple these individual cell types among each other. There is another network of interstitial cells of Cajal in the deep muscular plexus, which are connected to circular muscles through gap junctions [67, 80, 81]. These gap junctions may be

heteromeric and thus can consist of more than 1 connexin species. Specifically, Cx43 frequently colocalizes with Cx40 and Cx45 in interstitial cells of Cajal in the canine intestine [49]. Besides the gap junctional connexin pool, connexins, in casu Cx26, Cx32 and Cx43, also gather as functional hemichannels, as occurring at the basal pole of human intestinal epithelial cells [59]. In comparison with connexins, much less attention has yet been paid to pannexins in the intestine. One study showed Panx1 expression in all layers of the human colon, including mucosa, muscularis mucosa, submucosa and muscularis externa. Panx1 hereby is mainly found in enteric ganglia, blood vessel endothelium, erythrocytes, epithelial cells and goblet cells [14]. Another report described Panx2 production in epithelial cells of the small intestine and the colon [15] (Table 1).

Hepatic connexins and pannexins

As much as 5 connexin family members are detectable in liver (Table 1), among which Cx32 is the predominant one expressed by hepatocytes [34, 35, 37–43]. Cx43 is the major connexin species produced by nonparenchymal liver cells, including Kupffer cells, stellate cells and sinusoidal endothelial cells [82–85]. The latter 3 as well as hepatocytes also stain positive for Cx26 [83], while endothelial cells of the hepatic arteries and the portal vein express Cx37 and Cx40 [83, 85–87]. Quantitatively, however, most gap junctions are found between hepatocytes. They form so-called plaques that occupy about 3 % of the hepatocyte membrane surface [88]. Unlike Cx32, which is evenly distributed in the liver parenchyma, gap junctions consisting of Cx26 are mainly established by periportal hepatocytes [89, 90]. Hepatocellular Cx32 has many binding partners, including other junctional components, such as the tight junction building stone occludin [91] and mitochondrial proteins [92]. At the transcriptional level, tissue-specific expression of connexins is accomplished by differential promoter usage and tissue-enriched transcription factors [93]. In this light, hepatocellular Cx32 production depends on the binding of hepatocyte nuclear factor 1 alpha at its P1 gene promoter [94]. Epigenetic actions, including DNA methylation and histone modifications, also control connexin expression in liver cells [95, 96]. In addition, Cx43 was found to be regulated by specific microRNAs [97]. At a more downstream level, gap junction opening is controlled by posttranslational connexin changes. Connexin phosphorylation plays a critical role in this so-called gating process. Except for Cx26, all connexins are subject to phosphorylation, which may have a varying outcome [98]. With respect to the liver, Cx32 phosphorylation by protein kinase A enhances GJIC [99], while the same event mediated by protein kinase C results in protection against proteolysis [100]. Pannexin production in liver tissue has been poorly investigated thus far. Only a handful of reports demonstrated Panx1 expression in liver tissue, in particular produced by hepatocytes [16–20] and Kupffer cells [22]. Two studies showed the presence of Panx2 in mouse liver [15] and rat hepatocytes [21].

Connexin and pannexin channels in gastrointestinal and hepatic physiology

Physiological functions of connexin and pannexin signaling in the stomach

Experiments with rats of different age showed that Cx32 presence and gap junction number gradually increase during maturation of surface mucous cells in the stomach. This suggests that GJIC between surface mucous cells is a determinant of gastric cell differentiation and of gastric homeostasis in general [41]. Cx43-based gap junctions are thought to play an important role in the regulation of gastroduodenal motility [52]. Furthermore, gap junctional channels in gastric glands were found to support acid secretion [34]. A recent study in guinea pig revealed that a cAMP-dependent signal propagates intercellularly to induce coordinated secretion in the entire gastric gland [101]. As a matter of fact, gap junction activity in gastric epithelial cells is increased by cAMP [102] and is modulated by the beta adrenergic nervous system [103]. A number of reports have shown a cytoprotective function for Cx32-based gap junctions in the stomach. In this respect, ischemia–reperfusion stress [40] or acid-induced injury [42] combined with perfusion with octanol, a gap junction uncoupler, reduces Cx32-positive spots in rat stomach. This indicates that inhibition of gap junction activity weakens the barrier function of the gastric mucosa in combination with ischemia–reperfusion stress or acid-induced injury. Therefore, facilitation of GJIC may protect the gastric mucosal barrier function by potentiating cellular integrity [40, 42]. Cx32 gradually reappears during healing following ethanol-induced [104] or acetic acid-induced [105] gastric insults in rat. Although pannexins have been identified in gastric tissue, their potential physiological roles in the stomach remain to be established [15, 16].

Physiological functions of connexin and pannexin signaling in the intestine

Cx43-based gap junctions in the gut endoderm are indispensable for the transfer of signals that determine the establishment of left–right asymmetry from the node to the lateral plate mesoderm during embryogenesis [106, 107]. In the adult intestine, Cx26-related GJIC plays a role in

maintaining epithelial barrier function by affecting the production of tight junction proteins [108]. Although surrounded by some controversy [80, 109], gap junctions are also believed to be important for intestinal nerve transmission and pacing. This particularly holds true for gap junctions composed of Cx43 in interstitial cells of Cajal and smooth muscle cells, which control intestinal motility [66, 110]. In fact, ablation of Cx43 in smooth muscle cells of the murine intestine results in altered visceromotor responses and muscle contractility, a decrease in gastrointestinal transit time, increased neutrophil infiltration and thickening of the tunica muscularis. This not only points to clear-cut functions for Cx43 in intestinal physiology, but also in its morphology [111]. Recently, Cx43-based hemichannels have been found to mediate calcium responses in enteric glia of mouse colon, which equally is critical for modulating colonic motility and transit [76]. In vitro experiments using cocultures of rat colonic smooth muscle cells and lumbosacral dorsal root ganglion neurons showed the establishment of functional Cx43-based gap junctions between these 2 cell types. Furthermore, mechanistic stimulation of the myocytes increased intracellular calcium concentrations in the neurons, a signal triggered by IP_3, which moves between neurons via gap junctions [71]. Cx36 is also involved in intestinal nerve transmission. It is colocalized with nitric oxide synthase in the myenteric plexus of mouse colon, suggesting a role for gap junctions in inhibitory nitrergic enteric neuronal activity. This is further substantiated by the notion that Cx36 knockout mice exhibit modified spontaneous contractility properties and altered responses to electrical field stimulation and cholinergic agonists in the gut [112]. Besides conveying electrical signals, Cx43 represents an important component of the protective innate immune response of the intestinal epithelium. Activation of Toll-like receptor 2 indeed modulates Cx43 expression and increases GJIC in intestinal epithelial cells, thereby controlling their barrier function and restitution during acute and chronic inflammatory damage. This enhances mucosal homeostasis between commensals and hosts [113]. In addition, gap junction activity is critical for the establishment of oral tolerance by antigen-presenting cells in the intestine. Specifically, $CX3CR1^+$ macrophages take up fed antigens in the duodenum, which are subsequently transferred to $CD103^+$ dendritic cells via Cx43-based gap junctions for further antigen presentation [64]. Connexins also seem to stabilize intestinal vasculature, since Cx37 and Cx40 knockout mice exhibit hemorrhages in gastrointestinal tissue with pronounced blood vessel dilatation and congestion [114]. Although solid scientific evidence is currently lacking, channels composed of Panx1 have been proposed to control colonic motility, secretion and blood flow [14].

Physiological functions of connexin and pannexin signaling in the liver

Hepatic connexin expression patterns drastically alter during liver development. Early hepatic progenitor cells express Cx43 and switch to Cx26, but especially to Cx32, during differentiation into hepatocytes [115–117]. Both Cx26 and Cx32 become measurable in the late stages of gestation and culminate 1 week postpartum, with Cx26 being mainly located periportally [90]. This coincides with the establishment of the glucagon receptor zonation pattern, which is particularly present in perivenous hepatocytes [89]. Curiously, glucagon itself is mainly detectable in the periportal area and enhances Cx26 gene transcription [118]. Therefore, the Cx26 zonation pattern in the liver is thought to be controlled at the transcriptional level by hormonal stimuli [90, 118]. With respect to liver vasculature, Cx37 and Cx40 are expressed in early hepatic arteries and portal veins, while Cx43 is detected in portal veins, but not in hepatic veins, during fetal mouse liver development [87].

Gap junctions are indispensable for maintaining the metabolic competence of the adult liver. This has been well exemplified for glycogenolysis. Disintegration of glycogen to glucose is triggered by hormonal and neuronal stimuli and is predominantly performed by periportal hepatocytes [119, 120]. In fact, Cx32-based gap junctions between hepatocytes underlie the propagation of the glycogenolytic response from the periportal to the pericentral acinar pole by controlling the intercellular exchange of IP_3. The latter activates calcium release from the endoplasmic reticulum, subsequently causing calcium waves throughout the acinus [119, 121]. In support of this is the finding that Cx32 knockout mice show decreased blood glucose levels upon glycogenolytic stimulation [120, 122]. Similarly, Cx43-containing gap junctions facilitate the spread of calcium waves necessary for ductular secretion from biliary endothelial cells and thus bile formation [123, 124]. Biotransformation capacity also depends on the establishment of gap junctions consisting of Cx32 between hepatocytes. Chemical induction of cytochrome P450 1A1/2 and 2B1/2 in rat parallels the downregulation of pericentral Cx32 protein levels [125–127]. This is believed to reflect a defense mechanism to restrict the intercellular trafficking of reactive intermediates that have been generated in biotransformation reactions [125]. Furthermore, hepatocellular gap junctions govern several other vital functional processes in the liver, such as albumin secretion and ammonia detoxification [128].

During liver regeneration upon partial hepatectomy in rodents, increased GJIC is observed in the G1 phase of the cell cycle, followed by a steep decrease upon initiation of DNA synthesis. These alterations are reflected at the level

of Cx32 expression and to a lesser extent in Cx26 production, while Cx43 seems unchanged [129–131]. Although gap junctions are obviously involved in liver cell growth, their role still is a matter of debate. Indeed, inhibition of the p38 mitogen-activated protein kinase pathway prevents reduction in Cx32 expression, but does not affect hepatocyte proliferative activity in the regenerating rat liver [132]. This suggests that downregulation of GJIC may occur independently of liver cell proliferation. Hepatocellular proliferative activity was also found unaltered in the regenerating liver of Cx32 knockout mice, yet the extent of synchronous initiation and termination of DNA synthesis became decreased [131]. Based on this observation, GJIC seems permissive to hepatocyte cell cycling upon mitogenic stimulation, rather than furnishing a decisive liver cell growth signal. Thus, gap junction closure may support the functional segregation of metabolic pools in dividing liver cells from their quiescent neighbors to avoid homeostatic imbalance [129, 133]. In contrast to this is the view that gap junctions fulfill much more determinate functions in liver cell cycling, in particular by controlling the intercellular trafficking of critical growth mediators, such as cAMP [134, 135].

The vast majority of research efforts to elucidate the role of connexin signaling in liver cell death thus far have been performed in vitro. During the early stages of experimentally induced apoptosis in cultured liver cells, both Cx43 expression and gap junction activity are promoted. Most likely, transiently enhanced GJIC is necessary for the propagation of apoptotic signals, such as calcium ions. Upon further progression of cell death, gap junction activity deteriorates and disappears between apoptotic bodies. This may serve the reduction of the flux of toxic metabolites, such as nitric oxide and superoxide ions, and hence the protection of living cells [136]. Interestingly, accumulating evidence shows that connexin hemichannels, rather than gap junctions, are involved in liver cell death. Upon induction of Fas-mediated apoptosis in cultured primary hepatocytes, GJIC rapidly declines, which is associated with a decay of the gap junctional Cx32 protein pool. Simultaneously, levels of newly synthesized Cx32 protein increase and gather in a hemichannel configuration. This becomes particularly evident towards the end stages of the cell death process. Subsequent experiments showed that Cx32-based hemichannels support the apoptotic-to-necrotic transition in hepatocytes [29]. Furthermore, Cx43 signaling, also involving hemichannels, was found to facilitate the onset of spontaneous apoptosis in primary hepatocyte cultures [30]. Cx43 hereby interacts with mitochondrial proteins [137], as also described for hepatic Cx32 [92]. As a matter of fact, connexin hemichannels not only occur at the plasma membrane surface, but also reside at other subcellular locations, such as at mitochondria, where their functions have been linked to cell survival [92, 138–140]. In recent years, pannexin channels have been identified as mediators of apoptotic processes [141–143]. Panx1 is known to colocalize with the P_2X_7 receptor and to form a so-called death receptor complex. Stimulation of the P_2X_7 receptor, such as mediated by ATP, is believed to trigger the opening of Panx1-based channels [144–146]. Furthermore, Panx1 is a substrate for caspase cleavage, resulting in the formation of an open channel and the release of "find me" signals, including ATP and uridine triphosphate, at the earliest stages of cell death to recruit phagocytes [141, 143]. Although evidence is lacking thus far, there are indications that this also takes place in the liver [18, 20].

Conclusions and perspectives

It has become clear that connexins and pannexins are crucial to maintain digestive homeostasis. GJIC is indispensable during digestive embryogenesis [106, 107] and alterations in connexin expression have been described during gastric cell and hepatocyte differentiation [41], suggesting an important role in cell maturation. Cellular communication through gap junctions is equally a major driver of gastrointestinal homeostasis by controlling processes such as gastroduodenal [52] and gut motility [66, 110], gastric acid secretion [34], gastric cytoprotection [40, 42], intestinal epithelial barrier function [108] and intestinal innate immune defense [113]. Similarly, GJIC underlies critical hepatic functions, including xenobiotic biotransformation [125–127], glycogenolysis [119–122], bile secretion [123, 124], ammonia detoxification and plasma protein synthesis [128]. In contrast to gap junctions, connexin hemichannels and pannexin channels seem to be mainly, but not solely, involved in pathological processes [14, 26–30]. Nonetheless, some physiological functions have been attributed to these channels. In particular, connexin hemichannels are involved in the modulation of colon motility and transit [76], while pannexin channels seem to participate in the control of colonic motility, secretion and blood flow [14]. However, their functional relevance in digestive homeostasis largely remains to be established. A prerequisite to further clarify the role of connexin hemichannels and pannexin channels is the development and use of specific tools to study these particular channel types. In this context, most, if not all, of the presently used inhibitors of connexin hemichannels and pannexin channels also suppress gap junctions [147]. Great expectations now lie with peptides that reproduce sequences of the primary connexin and pannexin protein structure, as they suppress connexin hemichannel and pannexin channel activity, respectively, without affecting the GJIC

[148–150]. Further research using such tools will undoubtedly shed more light on the involvement of connexin and pannexin signaling in digestive homeostasis.

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