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Claudins in teleost fishes

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Abbreviations: AC, accessory cell; BBB, blood-brain barrier; BRB, blood-retina barrier; cldn(s), claudin(s); CNS, central nervous system; dpf, days post fertilization; EMT, epithelial-mesenchymal transition; FW, freshwater; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; GI, gastrointestinal; HRV, hyaloid-retinal vessel; IPW, ion-poor water; MRC, mitochondria-rich cell; ON, optic nerve; PVC, pavement cell; SW, seawater; TJ, tight junction; TER, transepithelial resistance; WGD, whole-genome duplication

Teleost fishes are a large and diverse animal group that represent close to 50% of all described vertebrate species. This review consolidates what is known about the claudin (Cldn) family of tight junction (TJ) proteins in teleosts. Cldns are transmembrane proteins of the vertebrate epithelial/endothelial TJ complex that largely determine TJ permeability. Cldns achieve this by expressing barrier or pore forming properties and by exhibiting distinct tissue distribution patterns. So far, ~63 genes encoding for Cldn TJ proteins have been reported in 16 teleost species. Collectively, *cldns* (or Cldns) are found in a broad array of teleost fish tissues, but select genes exhibit restricted expression patterns. Evidence to date strongly supports the view that Cldns play a vital role in the embryonic development of teleost fishes and in the physiology of tissues and organ systems studied thus far.

Introduction

Extant fishes are a large and diverse group of aquatic vertebrates numbering ~28,000 species.[1] This accounts for roughly 50% of all living vertebrates. Fishes within the class Actinopterygii (i.e., ray-finned fishes), belonging to the Division Teleostei, dominate the fish assemblage with ~27,000 members. These fishes are commonly referred to as teleosts, and of all the vertebrates, they can be considered the most diverse and most diversified taxon.[1] Teleosts are found in almost every conceivable aquatic habitat, ranging from polar seas at –2°C to highly alkaline hot springs at 45°C and from hadal depths of almost 8000 meters to high mountain lakes and streams. They are of tremendous economic importance as a food source as well as for recreational purposes.[2,3] In addition, (and in some measure because of the aforementioned diversity, plasticity and economic importance) teleosts have provided basic and applied scientists with countless experimental models, including some broadly known examples such as the zebrafish *Danio rerio*, medaka *Oryzias latipes* and the Japanese puffer fish *Takifugu* (= *Fugu*) *rubripes*. This review provides a timely report on what has been learned about the presence, distribution and function of claudin (Cldn) tight junction (TJ) proteins in teleost fishes since these integral components of the vertebrate TJ complex were first described in fishes just over a decade ago.

As one of the four basic tissue types in metazoans, epithelia are varied and exhibit an immensely dynamic and complex physiology. However, in its simplest form, the morphology of an epithelium can be broken down to a few basic components. That is, an epithelium is a sheet of interconnected and variously specialized transport cells lying atop an acellular basement membrane. In vertebrates, the cell-cell connections or junctional complex of an epithelium is a tripartite arrangement of elements that reside in a juxtaluminal position.[4] The apical-most constituent of the junctional complex is the TJ. The two main functions of the TJ are (1) to prevent the uncontrolled passage of solutes and water through the paracellular cleft between epithelial cells (i.e., TJ "gate" function) and (2) to confine membrane proteins of the epithelial cell to either the apical or basolateral domain which, in turn, will establish the correct configuration of membrane elements necessary for directional transcellular transport (i.e., TJ "fence" function).[5] The structure and function of the TJ complex in teleost fishes appears to be fundamentally similar to the structure and function of the TJ complex in other vertebrate groups (see ref. 6). More specifically, in teleosts, the TJ is generally accepted to act as a selectively permeable barrier that regulates the movement of solutes between fluid compartments. In addition, it seems very likely that the TJ also acts as a "fence" in teleosts, although there do not appear to be any studies that specifically address this. The TJ complex was first reported in a teleost fish by Öberg,[7] although around the same time that the TJ was first described in detail by Farquhar and Palade,[4] exquisite images of "leaky" TJs in the gill epithelium of a seawater (SW) acclimated teleost fish (*Fundulus similis*) were described by Philpott and Copeland.[8] However, these "leaky" TJs were not recognized as such, possibly because their morphology did not suggest occlusion of the intercellular space. A major breakthrough in the understanding of TJ function was the discovery of Cldn proteins.[9] It is now known that Cldns make up the greater part of the TJ complex architecture, and Cldns are generally considered to be the proteins

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primarily responsible for regulating the paracellular permeability properties of vertebrate epithelia.[5] Essentially this is because Cldns can be functionally divided into those that enhance the "barrier" properties of a TJ or those that enhance the "leak and/or pore" forming properties of a TJ.[5,10] In addition, the specific characteristics of a "barrier" or "pore/leak" forming Cldn can restrict or facilitate the movement of select solutes.[5,10] Therefore, the distinct expression patterns of Cldns in vertebrate epithelia/endothelia play a leading role in governing the paracellular permselectivity properties of a tissue. The first study to report a *cldn* in a teleost fish was conducted by Chin et al.[11] who used *cldn-7* as a distal foregut marker in zebrafish embryos. Shortly thereafter, Kollmar et al.[12] reported another 14 *cldns* in the zebrafish and discussed their importance in vertebrate morphogenesis. Since these first reports, ~63 *cldns* have been described in 16 different species of teleost fish. From the studies conducted to date, it is clear that members of the Cldn family of proteins play a crucial role in the development of teleost fishes as well as the physiological function of the epithelial and endothelial tissues examined thus far. Therefore, the current review provides a first consolidated overview of what is presently known about Cldns in teleost fishes. Because we focus our attention on the presence, distribution and function of Cldns in teleost fishes, we direct the reader to any number of excellent reviews for detailed information on the structure and function of vertebrate Cldns (e.g., see refs 5, 10, 13 and 14).

Claudin Diversity in Teleost Fishes

Evidence to date suggests that the genomes of teleost fishes possess large numbers of genes encoding for Cldns. This was definitively revealed by Loh et al.[15] using the puffer fish *Takifugu* (= *Fugu*) *rubripes*. In *Fugu*, 56 *cldns* have been described[15] and 35 can be assigned orthology to 17 mammalian *cldns*. At this stage, the remaining 21 *cldns* appear to be specific to the teleost fish lineage and have no mammalian counterparts.[15] It is proposed that the expansion of the claudin gene family in *Fugu* and other teleosts can be partly attributed to polyploidization or more specifically a whole-genome duplication (WGD) event that occurred near the base of the ray-finned fish (Actinopterygian) evolutionary tree.[15-19] In addition, it has also been proposed that multiple tandem gene duplications events in teleosts further contributed to the expansion of the claudin family following WGD, leading to several paralogues of the same *cldn* being found within a species.[15] Therefore, in tetrapods there may be two isoforms of the same *cldn* (e.g., human *CLDN*-10a and -10b), whereas in teleost fishes there may be up to five isoforms (e.g., *Fugu cldn-10a, -10b, -10c, -10d* and *-10e*).

Following a gene duplication event, new genes can either (1) be lost (i.e., become pseudogenes or silent mutations), (2) double the capacity of a crucial pathway or (3) persist and gain new function (i.e., neofunctionalization), if there is enough evolutionary pressure to drive the development of a new function.[20] Genomic studies of non-claudin genes have shown that while some teleost fish species (e.g., zebrafish) retain many duplicated genes, others (e.g., *Fugu*) have lost duplicated genes.[16,17] The latter does not appear to be the case with *cldns* and presumably neofunctionalization has played a role in the maintenance of an expanded *cldn* family in teleosts. As a result, related genes are often found to be expressed in different tissues (e.g., *Fugu cldn-11a* is expressed in the brain, heart, kidney and testis while *Fugu cldn-11b* is only found in the liver).[15] Additional knowledge in this area awaits further characterization of teleost *cldns*. However, why a large number of duplicated *cldns* have been maintained in teleost fishes (despite significant gene loss normally following WGD) is an important question. Put simply, why are there so many *cldns* in fishes? At this stage it could be speculated that because the diversity and success of teleost fishes as a group may be partly attributed to physiological plasticity, neofunctionalization of *cldns* and the diversification of tissue specific TJ properties may be a contributing factor. In this regard, it is worth noting that although teleost fishes possess epithelial tissues that function in a manner fundamentally similar to terrestrial vertebrates (e.g., in the kidney, etc.), other epithelia, such as those found in the skin and gill, are (in most cases) directly exposed to water throughout life. As tissue barriers that separate the internal milieu of the fish and an external environment that can (and often does) exhibit great variation in abiotic conditions, both the gill and the skin play a critical role in the maintenance of homeostasis. Not surprisingly, both of these tissues also possess a large complement of *cldns* and recent evidence suggests that many are exquisitely sensitive to environmental change [see section on Claudins in the teleost fish gill and on Claudins in the skin of teleosts]. Therefore it seems plausible that a large *cldn* family may have contributed to the success of teleosts animals by playing a role in physiological plasticity, which in turn contributed to the radiation of these fishes into a vast array of niches and adaptive zones. Indeed, given the diversity of teleost fishes, it is hard to imagine another group in the vertebrate lineage more suited for an increase in *cldn* diversity following gene duplication.

Claudin Nomenclature in Teleost Fishes

Nomenclature of *cldns* in teleost fishes follows two sets of rules. For zebrafish *cldns*, each has been named after its human ortholog (e.g., *cldn-11*, *cldn-7*, etc).[12] However, when no unambiguous *cldn* ortholog could be found, a character suffix was assigned to a *cldn* (e.g., *cldn-a*, *cldn-b*, etc.).[12] As our knowledge has developed, it could be rationalized that character suffixes in the absence of a numerical designation are becoming increasingly redundant. For example, sequence analysis of zebrafish *cldn-h* now shows that it is equivalent to *cldn-3a* in a variety of other teleost species. Nevertheless, single character suffixes continue to be used and have been adopted in species of teleost fish closely related to zebrafish (e.g., goldfish, *Carassius auratus*).[21-23] In *Fugu*, *cldns* have been assigned a numerical designation, and where possible this was in accord with their mammalian (human) counterparts,[15] whereby genes were given the same names as their human orthologs. Duplicate copies of a *cldn* that had already received a numerical designation were then designated an additional letter suffix (e.g., *cldn-3a, -3b, -3c* and *-3d*) and novel genes (i.e., those with no human ortholog) were numbered starting with *cldn-25*

in an identical manner.[15] This latter "*Fugu* convention" has been followed in almost all species of teleost fish where *cldns* have been described (see **Table 1**). In this review we will adhere to nomenclature adopted in the literature cited, be it a single character or numerical suffix. However, when a single character suffix is used, in parenthesis we will endeavor to provide the reader with a numerical suffix that corresponds to the *cldn* as it is known in other teleost species (e.g., *cldn-h* = *cldn-3a*).

Tissue-Specific Claudin Expression in Teleost Fishes

As in other vertebrates, *cldns* in teleost fishes are expressed in a tissue specific manner. Also, in accord with observations of Cldns in other vertebrates, cldns in fishes are found to vary in abundance between tissues or between different regions of the same tissue. In some cases *cldns* are reported to be exclusive to select tissues (e.g., see ref. 15). A summary of *cldn* presence in different teleost fish tissues is presented in **Table 1** and the following sections consider the many roles that *cldns*/Cldns are either currently known, or proposed, to play in teleost fish tissues. However, it is worth noting that there is little to no functional insight into a great many Cldns found in teleost fishes. Therefore, where possible, each of the following sections contains a summary of *cldns* that are reported to be present in a teleost fish tissue by expression profiling, but have not yet received attention with respect to possible function. This may provide an impetus for further study.

Claudins and teleost development. Many members of the claudin family are reported to play crucial roles in the embryonic development of vertebrates.[24] During zebrafish embryonic development, it has been proposed that *cldn-e* (= *cldn-28b*) is required for epiboly, a process that jump-starts tissue differentiation in a developing embryo and involves extensive migration of cell layers.[25] Knockdown of *cldn-e* in developing zebrafish embryos significantly delayed epiboly which resulted in most embryos dying by the end of gastrulation. Therefore it was suggested that the presence of a functional Cldn-e protein is crucial for the progression of epiboly and successful gastrulation in zebrafish.[25] Mutations in zebrafish *cldn-j* (= *cldn-6*) cause defects in otolith formation as well as vestibular and hearing dysfunction.[26] Because *cldn-j* is expressed in the otic vesicle early in the critical period of otolith growth, and mutant embryos exhibit a significant decline in *cldn-j* mRNA, a hypomorphic or null effect in *cldn-j* is suggested.[26] Nevertheless, the otic placode appears to form and cavitate normally, therefore it is not entirely clear how otolith formation is compromised in *cldn-j* mutants. The authors hypothesize that a deleterious effect on barrier or signaling function could play a role.[26] In addition, the absence of *cldn-b* (= *cldn-30d*) has also been reported during impaired inner-ear development in zebrafish embryos.[27]

In newly developing zebrafish embryos, *cldn-7* has been shown to mark the earliest stages of gut development,[11] and *cldn-c* (= *cldn-3d*) is also thought to be involved in the development of the zebrafish gastrointestinal (GI) tract.[28] In zebrafish embryos, *cldn-c* is preferentially expressed in the GI tract at an early stage of development and is involved in the formation and thickening of the endodermal rod between 1–2 d post fertilization (dpf).[28] As gut development proceeds, *cldn-c* persists (i.e., through all developmental stages including lumen formation, intestinal cell differentiation, epithelial folding and gut motility by 5 dpf), and it is believed to be involved in transmembrane signaling during stratification of the intestinal epithelium.[28] In the later stages of zebrafish embryonic gut development (i.e., lumen formation), *cldn-15* is also proposed to be involved.[29] Zebrafish *cldn-15* exhibits the hallmarks of a "leaky" claudin as its overexpression in LLC-PK1 or MDCKC7 cells has been shown to reduce transepithelial resistance (TER) in both cases.[29] Interestingly, zebrafish *cldn-15* shares high sequence similarity to human *CLDN-10*, the latter of which forms either anion pores (e.g., CLDN-10a) or cation pores (e.g., CLDN-10b).[30] Similar to human CLDN-10, zebrafish Cldn-15 also serves as a pore forming TJ protein, although the ion selectivity characteristics of Cldn-15 are not yet determined.[29] During intestinal lumen development, the presence of pore forming Cldn-15 appears to be essential. Under the control of transcription factor Tcf2, "leaky" Cldn-15 provides a paracellular pore for ion movement and consequently increases luminal fluid accumulation and volume expansion in multiple small lumens. This promotes coalescence of the small lumens to successfully form one single intestinal lumen that persists through adulthood.[29] The development of other regions within the gastrointestinal system in teleost fishes also appears to rely on *cldn-15* or at least *cldn-15* like isoforms. Recently it has been reported that *cldn 15-like b* (*cldn-15lb*) plays a role in hepatocyte polarization and biliary duct morphogenesis in zebrafish.[31] In these studies, *cldn-15lb* mutants revealed hepatocyte polarization defects, canalicular malformations as well as a disorganized biliary duct network.[31]

Not surprisingly, much of the elegant work conducted on the role(s) of various *cldns* in the development of teleost fishes uses zebrafish, which is one of the premier animal models in the developmental field. However, early studies using transgenic medaka (*Oryzias latipes*) possessing *cldn-7* fused to enhanced green fluorescent protein (GFP) allowed the first in vivo observations of TJ dynamics during the course of embryogenesis in a living animal.[32] In medaka embryos, *cldn-7* was found to be expressed in the pronephric duct, otic vesicle, olfactory primordium and skin at stage 23 (~1 d and 17 h post fertilization) and at 2 dpf, *cldn-7* was found localized to cell-cell junctions in the retina, neural tube and the skin.[32] More recently, a *cldn-k* fused GFP zebrafish model has been used to study myelination during development.[33] In the central nervous system *cldn-k* mRNA and protein expression was observed 2 and 3 dpf respectively.[33] More specifically, *cldn-k* was found in regions consistent with localization in autotypic TJs of oligodendrocytes and myelinating Schwann cells of the hindbrain starting at 3 dpf and progressing to the adult retinal oligodendrocytes as well as other myelinated structures in adult zebrafish.[33] In addition, *cldn-k* has also been reported to be present in Schwann cells associated with the lateral-line system and the spinal cord at 4 dpf.[34] The presence of *cldn-k* in the central and peripheral nervous systems of zebrafish is related to the origin of these structures from the same primordia and can be traced during embryonic development.

Table 1. Claudin expression in discrete tissues of teleost fishes

Claudin	Species	Tissue expression	References
Claudin-1	*Astatotilapia burtoni*	ON	Mack and Wolburg (2006)
	Danio rerio (previously *cldn-19*)	Br, Eye, Gill, He, Liv, Kid, Mus, Sk, Ov, Tes	Vihtelic et al. (2005); Clelland and Kelly (2010a, 2011); Kumai et al. (2011)
	Takifugu rubripes	Eye, Gill, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-2	*Cyprinus carpio*	Br, Gill, Int, Liv, Sp, Kid, Sk	Syakuri et al. (2013)
	Danio rerio	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Ov, Tes	Clelland and Kelly (2010a); Kumai et al. (2011)
	Takifugu rubripes	Br, Eye, He, Liv, Kid	Loh et al. (2004)
Claudin-3	*Astatotilapia burtoni*	ON	Mack and Wolburg (2006)
	Danio rerio	Br	Grupp et al. (2010)
	Dicentrarchus labrax	Gill, Int	Boutet et al. (2006)
	Fundulus heteroclitus	Gill	Whitehead et al. (2011)
	Oreochromis mossambicus	Gill	Tipsmark et al. (2008a)
	Paralichthys lethostigma	Gill	Tipsmark et al. (2008c)
Claudin-3a	*Carassius auratus* (= *cldn-h*)	Gill, Int, Liv, Gb, Sb, Kid, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Danio rerio (= *cldn-h*)	Eye, Gill, Int, Kid, Ov, Tes	Clelland and Kelly (2010a, 2011); Kumai et al. (2011)
	Oncorhynchus mykiss	Gill	Chasiotis and Kelly (2011); Kelly and Chasiotis (2011)
	Salmo salar	Br, Eso, PC, Int, Liv, Kid	Tipsmark and Madsen (2012)
	Takifugu rubripes	He, Int, Kid	Loh et al. (2004)
	Tetraodon biocellatus	Gill, Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Gill, He, Int, Liv, Kid, Sk, Ov, Tes	Bagherie-Lachidan et al. (2008); Bui et al. (2010); Clelland and Kelly (2010b); Pinto et al. (2010); Bui and Kelly (2011)
Claudin-3b	*Cyprinus carpio*	Br, Gill, Int, Liv, Sp, Kid, Sk	Syakuri et al. (2013)
	Salmo salar	Br, Gill, Eso, PC, Int, Liv, Kid, Mus,	Tipsmark and Madsen (2012)
	Takifugu rubripes	Br, Eye, He, Int, Liv, Kid, Sk	Loh et al. (2004)
	Tetraodon biocellatus	Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, He, Int, Liv, Kid, Ov, Tes	Bagherie-Lachidan et al. (2008); Clelland and Kelly (2010b)
Claudin-3c	*Cyprinus carpio*	Br, Gill, Int, Liv, Sp, HKid, Kid, Sk,	Syakuri et al. (2013)
	Salmo salar	Br, Gill, Eso, PC, Int, Kid	Tipsmark and Madsen (2012)
	Tetraodon biocellatus	Gill, Kid	Duffy et al. (2011)
	Takifugu rubripes	Br, Eye, Gill, Int, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Int, Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2008); Bui et al. (2010); Clelland and Kelly (2010b); Bui and Kelly (2011)
Claudin-3d	*Carassius auratus* (= *cldn-c*)	Gill, Int, Liv, Gb, Sb, Kid	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Danio rerio (= *cldn-c*)	He, Int, Liv, Kid, Ov, Em (Int, Liv, Pa)	Stuckenholz et al. (2009); Clelland and Kelly (2010a); Kumai et al. (2011)
	Takifugu rubripes	Int, Kid	Loh et al. (2004)
	Tetraodon biocellatus	Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Int, Kid	Bagherie-Lachidan et al. (2008); Clelland et al. (2010b)
Claudin-4	*Fundulus heteroclitus*	Gill	Whitehead et al. (2011)

Br, Brain; BBB, Blood-Brain Barrier; VS, Vascular System; ON, Optic Nerve; OT, Otic Vesicle; NT, Nervous Tissue; He, Heart; Eso, Esophagus; PC, Pyloric Ceca; Int, Intestine; Liv, Liver; GB, Gall Bladder; Pa, Pancreas; Sb, Swim Bladder; Sp, Spleen; Kid, Kidney; HKid, Head Kidney; Mus, Muscle; Sk, Skin; Ov, Ovary; Tes, Testis; Em, Embryo; WB, Whole Body; EVL, Enveloping Layer; Som, Somites; OP, Olfactory Placode; LL, Lateral Line.

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	Oreochromis mossambicus	Gill	Tipsmark et al. (2008a)
	Paralichthys lethostigma	Gill	Tipsmark et al. (2008c)
Claudin-5	*Danio rerio*	Br (BBB), Em (Br, BBB, NT, VS)	Jin et al. (2005); Jeong et al. (2008); Zhang et al. (2010); Zheng et al. (2010); Xie et al. (2010); Hyoung Kim et al. (2011)
Claudin-5a	*Takifugu rubripes*	Br, Eye, Gill, Int, Kid	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-5b	*Takifugu rubripes*	Br, Eye, Gill, He, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-5c	*Takifugu rubripes*	Em	Loh et al. (2004)
Claudin-6	*Danio rerio* (= *cldn-j*)	Br, Ov, Em (Br, OT)	Hardison et al. (2005); Clelland and Kelly (2010a); Han et al. (2011); Kumai et al. (2011)
	Takifugu rubripes	Gill, He, Int, Liv	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui et al. (2010); Bui and Kelly (2011)
Claudin-7	*Carassius auratus*	Br, Eye, Gill, Int, Liv, Gb, Sb, Kid, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Danio rerio	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Ov, Tes, Em (OT, Int)	Chin et al. (2000); Vihtelic et al. (2005); Clelland and Kelly (2010a); Han et al. (2011); Kumai et al. (2011)
	Oncorhynchus mykiss	Gill	Chasiotis and Kelly (2011); Kelly and Chasiotis (2011)
	Cyprinus carpio	Br, Gill, Int, Liv, Sp, HKid, Kid, Sk	Adamek et al. (2013); Syakuri et al. (2013)
	Oryzias latipes	Em (Sk, Eye, Kid, NT, OT)	Miyamoto et al. (2009)
Claudin-7a	*Takifugu rubripes*	Br, Eye, Gill, He, Int, Liv, Kid, Sk, Ov, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-7b	*Takifugu rubripes*	Br, Eye, Gill, He, Kid, Mus, Tes	Loh et al. (2004)
Claudin-8	*Danio rerio*	Eye, Gill, He, Int, Kid, Sk, Ov, Tes	Clelland and Kelly (2010a); Kumai et al. (2011)
Claudin-8a	*Takifugu rubripes*	Br, Eye, Gill, Sk, Tes	Loh et al. (2004)
	Tetraodon biocellatus	Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Int, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2009); Bui and Kelly (2011)
Claudin-8b	*Takifugu rubripes*	Eye, Gill, Int, Kid, Sk	Loh et al. (2004)
	Tetraodon biocellatus	Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2009); Bui and Kelly (2011)
Claudin-8c	*Takifugu rubripes*	Gill, He, Int, Kid, Sk	Loh et al. (2004)
	Tetraodon biocellatus	Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Int, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2009); Bui and Kelly (2011)
Claudin-8d	*Carassius auratus*	Br, Eye, Gill, Int, Liv, Gb, Sb, Kid, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Oncorhynchus mykiss	Gill	Chasiotis and Kelly (2011); Kelly and Chasiotis (2011)
	Takifugu rubripes	Gill, Sk	Loh et al. (2004)
	Tetraodon biocellatus	Kid	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Int, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2009); Bui et al. (2010); Pinto et al. (2010); Bui and Kelly (2011)
Claudin-10	*Danio rerio*	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Ov, Tes	Clelland and Kelly (2010a)
	Hypomesus transpacificus	WB	Connon et al. (2011)
Claudin-10b	*Takifugu rubripes*	Eye, Kid	Loh et al. (2004)
Claudin-10c	*Takifugu rubripes*	Eye, Gill, Sk	Loh et al. (2004)
Claudin-10d	*Takifugu rubripes*	Int	Loh et al. (2004)

Br, Brain; BBB, Blood-Brain Barrier; VS, Vascular System; ON, Optic Nerve; OT, Otic Vesicle; NT, Nervous Tissue; He, Heart; Eso, Esophagus; PC, Pyloric Ceca; Int, Intestine; Liv, Liver; GB, Gall Bladder; Pa, Pancreas; Sb, Swim Bladder; Sp, Spleen; Kid, Kidney; HKid, Head Kidney; Mus, Muscle; Sk, Skin; Ov, Ovary; Tes, Testis; Em, Embryo; WB, Whole Body; EVL, Enveloping Layer; Som, Somites; OP, Olfactory Placode; LL, Lateral Line.

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	Tetraodon nigroviridis	Gill	Bui et al. (2010); Bui and Kelly (2011)
Claudin-10e	*Tetraodon nigroviridis*	Gill	Bui et al. (2010); Bui and Kelly (2011)
	Salmo salar	Br, Gill, He, Int, Liv, Kid, Mus	Tipsmark et al. (2008b, 2009)
Claudin-11	*Astatotilapia burtoni*	ON	Mack and Wolburg (2006)
	Cyprinus carpio	Br, Gill, Int, Liv, Sp, HKid, Kid, Sk	Syakuri et al. (2013)
	Danio rerio	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Sk, Ov, Tes	Clelland and Kelly (2010a); Kumai et al. (2011)
Claudin-11a	*Takifugu rubripes*	Br, He, Kid, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui et al. (2010); Bui and Kelly (2011)
Claudin-11b	*Takifugu rubripes*	Liv	Loh et al. (2004)
Claudin-12	*Carassius auratus*	Br, Eye, Gill, Int, Liv, Gb, Sb, Kid, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Danio rerio	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Ov, Tes	Vihtelic et al. (2005); Clelland and Kelly (2010a, 2011); Kumai et al. (2011)
	Oncorhynchus mykiss	Gill	Chasiotis and Kelly (2011); Kelly and Chasiotis (2011)
	Takifugu rubripes	Br, Eye, Gill, He, Int, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-13	*Takifugu rubripes*	Gill	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-14			
Claudin-14b	*Takifugu rubripes*	Eye, Gill, He, Int, Liv, Kid, Tes	Loh et al. (2004)
Claudin-15	*Danio rerio*	Gill, Int, Kid, Ov, Tes, Em (Int, Kid)	Bagnat et al. (2007); Clelland and Kelly (2010a)
	Salmo salar	PC, Int	Tipsmark et al. (2010)
Claudin-15a	*Takifugu rubripes*	Int, Kid	Loh et al. (2004)
Claudin-15b	*Danio rerio* (= *claudin-15-like b*)	Em (Liv, Int, Pa)	Cheung et al. (2012)
Claudin-19	*Takifugu rubripes*	Eye, Gill	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-20			
Claudin-20a	*Takifugu rubripes*	Br, He	Loh et al. (2004)
Claudin-23	*Cyprinus carpio*	Br, Gill, Int, Sp, Kid, Sk	Adamek et al. (2013); Syakuri et al. (2013)
Claudin-23a	*Takifugu rubripes*	Br, Eye, Gill, He, Int, Liv, Kid, Sk, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-23b	*Takifugu rubripes*	Int, Mus	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui et al. (2010); Bui and Kelly (2011)
Claudin-25	*Takifugu rubripes*	Eye, Int	Loh et al. (2004)
Claudin-25a	*Salmo salar*	PC, Int	Tipsmark et al. (2010)
Claudin-25b	*Salmo salar*	PC, Int, Liv	Tipsmark et al. (2010)
Claudin-26	*Takifugu rubripes*	Br, Eye, Gill, He, Int, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Loh et al. (2004)
Claudin-27			
Claudin-27a	*Salmo salar*	Br, Gill, He, Int, Liv, Kid, Mus	Tipsmark et al. (2008b, 2009)
	Takifugu rubripes	Eye, Gill, Kid, Sk	Loh et al. (2004)
	Tetraodon biocellatus	Gill	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, Int, Liv, Sp, Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2009); Bui et al. (2010); Bui and Kelly (2011)

Br, Brain; BBB, Blood-Brain Barrier; VS, Vascular System; ON, Optic Nerve; OT, Otic Vesicle; NT, Nervous Tissue; He, Heart; Eso, Esophagus; PC, Pyloric Ceca; Int, Intestine; Liv, Liver; GB, Gall Bladder; Pa, Pancreas; Sb, Swim Bladder; Sp, Spleen; Kid, Kidney; HKid, Head Kidney; Mus, Muscle; Sk, Skin; Ov, Ovary; Tes, Testis; Em, Embryo; WB, Whole Body; EVL, Enveloping Layer; Som, Somites; OP, Olfactory Placode; LL, Lateral Line.

Table 1. Claudin expression in discrete tissues of teleost fishes

Claudin-27b	*Danio rerio* (= *cldn-f*)	Eye, Gill, Ov, Em (WB)	Clelland and Kelly (2010a); Kumai et al. (2011); Vesterlund et al. (2011)
	Takifugu rubripes	Eye, Gill, Int, Liv, Kid, Sk	Loh et al. (2004)
	Tetraodon biocellatus	Gill	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Int, Liv Kid, Mus, Sk, Ov, Tes	Bagherie-Lachidan et al. (2009); Bui and Kelly (2011)
Claudin-27c	*Anguilla anguilla*	Gill	Kalujnaia et al. (2007)
	Takifugu rubripes	Em	Loh et al. (2004)
	Tetraodon biocellatus	Gill	Duffy et al. (2011)
	Tetraodon nigroviridis	Br, Eye, Gill, He, Mus, Sk	Bagherie-Lachidan et al. (2009); Bui et al. (2010); Bui and Kelly (2011)
Claudin-27d	*Takifugu rubripes*	Br, Gill	Loh et al. (2004)
	Tetraodon nigroviridis	Br, Eye, Gill, Mus, Sk, Tes	Bagherie-Lachidan et al. (2009); Bui and Kelly (2011)
Claudin-28			
Claudin-28a	*Oreochromis mossambicus*	Gill	Tipsmark et al. (2008a)
	Salmo salar	Br, Gill, He, Int, Liv, Kid, Mus	Tipsmark et al. (2008b, 2009)
	Takifugu rubripes	Eye, Gill, He, Kid, Mus, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Pinto et al. (2010); Bui and Kelly (2011)
Claudin-28b	*Danio rerio* (= *cldn-e*)	Br, Eye, Gill, He, Kid, Mus, Sk, Ov, Em (EVL, Sk, OT, OP)	Vihtelic et al. (2005); Clelland and Kelly (2010a); Siddiqui et al. (2010); Kumai et al. (2011)
	Carassius auratus (= *cldn-e*)	Eye, Gill, Int, Liv, Sb, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Oncorhynchus mykiss	Gill	Chasiotis and Kelly (2011); Kelly and Chasiotis (2011); Sandbichler et al. (2011)
	Salmo salar	Br, Gill, He, Int, Liv, Kid, Mus	Tipsmark et al. (2008b, 2009)
	Takifugu rubripes	Gill, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-28c	*Takifugu rubripes*	Eye, Gill, He, Int, Kid, Sk, Ov, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-29			
Claudin-29a	*Carassius auratus* (= *claudin-d*)	Br, Eye, Gill, Int, Liv, Gb, Sb, Kid, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Danio rerio (= *claudin-d*)	Br, Eye, Gill, He, Int, Kid, Mus, Ov, Tes, Em (WB)	Clelland and Kelly (2010a, 2011); Kumai et al. (2011); Vesterlund et al. (2011)
	Takifugu rubripes	Ov, Tes	Loh et al. (2004)
Claudin-29b	*Takifugu rubripes*	Br, Gill, Int, Ov, Tes	Loh et al. (2004)
Claudin-30	*Cyprinus carpio*	Br, Gill, Int, Liv, Sp, HKid, Kid, Sk	Adamek et al. (2013); Syakuri et al. (2013)
	Oncorhynchus mykiss	Gill	Chasiotis and Kelly (2011); Kelly and Chasiotis (2011)
	Oreochromis mossambicus	Gill	Tipsmark et al. (2008a)
	Salmo salar	Br, Gill, He, Int, Liv, Kid, Mus	Tipsmark et al. (2008b, 2009); Engelund et al. (2012)
Claudin-30a	*Takifugu rubripes*	Eye, Gill, He, Int, Liv, Kid, Mus, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-30b	*Takifugu rubripes*	Eye, Gill, He, Liv, Mus, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-30c	*Takifugu rubripes*	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)

Br, Brain; BBB, Blood-Brain Barrier; VS, Vascular System; ON, Optic Nerve; OT, Otic Vesicle; NT, Nervous Tissue; He, Heart; Eso, Esophagus; PC, Pyloric Ceca; Int, Intestine; Liv, Liver; GB, Gall Bladder; Pa, Pancreas; Sb, Swim Bladder; Sp, Spleen; Kid, Kidney; HKid, Head Kidney; Mus, Muscle; Sk, Skin; Ov, Ovary; Tes, Testis; Em, Embryo; WB, Whole Body; EVL, Enveloping Layer; Som, Somites; OP, Olfactory Placode; LL, Lateral Line.

Table 1. Claudin expression in discrete tissues of teleost fishes

Claudin-30d	*Carassius auratus* (= *claudin-b*)	Eye, Gill, Int, Liv, Gb, Sb, Kid, Sk	Chasiotis and Kelly (2011, 2012); Chasiotis et al. (2012)
	Danio rerio (= *claudin-a*)	Br, Eye, Gill, He, Kid, Mus, Ov, Em (OT)	Kollmar et al. (2001); Vihtelic et al. (2005); Clelland and Kelly (2010a); Han et al. (2011); Kumai et al. (2011)
	Danio rerio (= *claudin-b*)	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Sk, Ov, Tes, Em (OT, OP, Kid)	Kollmar et al. (2001); Vihtelic et al. (2005); Han et al. (2011); Kumai et al. (2011); Clelland and Kelly (2010a); Kwong et al. (2013)
	Takifugu rubripes	Eye, Gill, He, Int, Kid, Mus, Sk, Ov, Tes	Loh et al. (2004)
Claudin-31	*Danio rerio* (= *claudin-g*)	Gill, He, Int, Liv, Kid, Mus, Ov, Tes, Em (WB, Som)	Sumanas et al. (2005); Qian et al. (2005); Clelland and Kelly (2010a, 2011); Kumai et al. (2011); Vesterlund et al. (2011)
	Danio rerio (= *claudin-k*)	Eye, ON, Em (Br, NS, LL)	Takada and Appel (2010); Münzel et al. (2012)
	Oncorhynchus mykiss	Gill	Kelly and Chasiotis (2011)
	Takifugu rubripes	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Sk, Tes	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui and Kelly (2011)
Claudin-32			
Claudin-32a	*Danio rerio* (= *claudin-i*)	Br, Eye, Gill, He, Int, Kid, Mus, Sk, Ov	Vihtelic et al. (2005); Clelland and Kelly (2010a); Kumai et al. (2011)
	Oncorhynchus mykiss	Gill	Kelly and Chasiotis (2011)
	Takifugu rubripes	Eye, Sk	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui et al. (2010); Pinto et al. (2010); Bui and Kelly (2011)
Claudin-32b	*Takifugu rubripes*	Br, Eye, Gill, He, Int, Liv, Kid, Mus, Sk, Tes	Loh et al. (2004)
Claudin-33			
Claudin-33b	*Takifugu rubripes*	Gill	Loh et al. (2004)
	Tetraodon nigroviridis	Gill	Bui et al. (2010); Bui and Kelly (2011)
Claudin-33c	*Takifugu rubripes*	Em	Loh et al. (2004)

Br, Brain; BBB, Blood-Brain Barrier; VS, Vascular System; ON, Optic Nerve; OT, Otic Vesicle; NT, Nervous Tissue; He, Heart; Eso, Esophagus; PC, Pyloric Ceca; Int, Intestine; Liv, Liver; GB, Gall Bladder; Pa, Pancreas; Sb, Swim Bladder; Sp, Spleen; Kid, Kidney; HKid, Head Kidney; Mus, Muscle; Sk, Skin; Ov, Ovary; Tes, Testis; Em, Embryo; WB, Whole Body; EVL, Enveloping Layer; Som, Somites; OP, Olfactory Placode; LL, Lateral Line.

Claudins in teleost nervous tissue, the blood-brain barrier and the eye

A large number of *cldns* have been reported to be present in the nervous and ocular tissues of teleost fishes (see **Table 1**). In *Fugu* alone, 19 *cldns* are found in the brain (*cldn-2, -3b, -3c, -5a, -5b, -7a, -7b, -8a, -11a, -12, -19, -20a, -23a, -26, -27d, -29b, -30c, -31* and *-32b*) and 28 *cldns* are present in the eye (*cldn-1, -2, -3b, -3c, -5a, -5b, -7a, -7b, -8a, -8b, -10b, -10c, -12, -14b, -23a, -25, -26, -27a, -27b, -28a, -28c, -30a, -30b, -30c, -30d, -31, -32a and -32b*).[45] Indeed, when considering all species of teleosts studied to date, it is simpler to list the *cldns* that are absent from nervous and ocular tissue. Specifically, *cldn-3d, -8* (in zebrafish), *-10b, -10c, -10d, -11b, -13, -14b, -15* (in zebrafish), *-15a, -15b, -25a, -25b, -28c, -30a, -30b and -33b* have either not been reported in or have been reported as absent from nervous tissue in teleosts studied to date (see **Table 1**). In ocular tissue, *cldn-3d, -5* (in zebrafish), *-6* (zebrafish *cldn-j*), *-10d, -10e, -11a, -11b, -13, -14b, -15* (in zebrafish), *-15a, -15b, -20a, -23, -23b, -25a, -25b, -30, -33b* and zebrafish *cldn-g* have either not been reported in or have been reported absent in teleosts studied so far (see **Table 1**). Therefore, of the ~63 *cldns* reported in all teleost species thus far, a conservative estimate indicates that ~70% are present either in the brain or in the eye (see **Table 1**). It would seem that *cldns* play an important role in the function of these tissues.

In the central and peripheral nervous systems of fishes, Cldns have been implicated in the development and maintenance of the blood-brain barrier (BBB). The BBB separates the extracellular fluid of the central nervous system (CNS) from blood and is necessary for protecting the neural microenvironment.[35] Capillary endothelial cells and surrounding astrocytes form the BBB of vertebrates and TJs can be found between adjacent endothelial cells. The presence of a functional BBB as early as 3 dpf in zebrafish embryos has been reported[36,37] and *cldn-5a* and *-5b* expression coincides with the formation of both the BBB and the blood-retina barrier (BRB) at around this time.[38,39] In adult zebrafish, *cldn-5a* and *-5b* have been immunolocalized to endothelial cells in the brain, and more specifically to endothelial cell boundaries of blood vessels in the brain, but not trunk blood vessels.[36,37] In addition to BBB/BRB formation, *cldn-5a* has been implicated in brain ventricular lumen expansion.[40] This process relies on fluid accumulation and hydrostatic pressure and *cldn-5a* has been identified as a key component in the development of the cerebral-ventricular barrier.[39,40] This is a crucial step in brain morphogenesis that precedes the establishment of the BBB.[39,40]

In addition to *cldn-5*, a Cldn-3 protein has been detected in the astroglial fibers of the tectum and telencephalon of the zebrafish brain,[41] where its function was suggested not to be confined to TJ formation, but perhaps to contribute to microenvironment formation by astroglial cells. Both Cldn-3 and -5 have been detected in the BBB of mammals[42] and as a result of recent developments in *cldn-5* research in zebrafish, this organism has been suggested as a vertebrate model for hemorrhaging stroke.[43]

Generally speaking, the BRB separates parts of the nervous system from the bloodstream supplying them, and as such is an extension or a subtype of the BBB.[37] Given that teleost *cldn-5* isoforms have been demonstrated to be important for maintaining the integrity of the BBB (see section above), it should come as no surprise that *cldn-5* isoforms are important in the maintenance of the BRB as well.[37] Both *cldn-5a* and *-5b* were detected in hyaloid-retinal vessels (HRVs, vessels that supply blood to the developing lens of an embryo and fully regress before hatching) and the outer membrane of the retina of the developed eye.[37,44] In the HRV however, Cldn-5 protein could not be correlated with BRB properties as the structure was found to be fairly leaky to a variety of fluorescent tracers.[44] The absence of a functional BRB in the HRV, in addition to the absence of ZO-1 and occludin, may suggest that Cldn-5 is not involved in the BRB formation, but instead, is involved in the establishment of the microenvironment similar to that of the optic nerve in tilapia.[35]

In the teleost fish optic nerve (ON), axons are continuously generated from new retinal ganglion cells and protrude toward the optic tectum, the part of the brain that is responsible for sensory-motor processing.[35] Teleost fish astrocytes surround these axons and are interconnected by desmosomes and TJs.[35] In the ON of tilapia (*Astatotilapia burtoni*), Cldn-1 was detected as part of the TJ complex interconnecting astrocytes.[35] Cldn-1 immunoreactivity was present in new unmyelinated neurons and co-localized with the astrocytic processor marker glial fibrillary acidic protein (GFAP), while Cldn-11 was detected in myelinated portions of the optic nerve.[35] The presence of TJs and Cldns within the teleost fish ON suggests that they may play a role in establishing fluid compartments of differing content that perhaps, promote growth of new unmyelinated axons.

Claudins in the teleost fish gill

The gill is an architecturally complex, multifunctional organ that plays a central role in teleost fish respiration, osmoregulation, acid/base balance and nitrogenous waste excretion.[45-47] The gill stroma is a heterogeneous epithelium that directly interfaces with the surrounding environment. Because the external environment (i.e., water) differs greatly from the internal milieu of the animal (i.e., blood/extracellular fluid), the gill epithelium is a vital and dynamic tissue barrier that is essential for the maintenance of homeostasis in teleost fishes. For a number of decades it has been broadly accepted that differing paracellular properties of the gill epithelium contribute to the function of this tissue (e.g., see refs. 48–50), but the molecular physiology of the gill TJ complex has only recently become a focus of attention. In this regard, studies on the gill epithelium TJ complex and its molecular components have been reviewed by Chasiotis et al.[6] Therefore the following section is not exhaustive, but rather, emphasizes salient points. We direct the reader to Chasiotis et al.[6] for an in-depth review of TJ proteins in the gills of fishes.

In *Fugu*, 32 of the 56 *cldns* described by Loh et al.[15] were found in gill tissue and similarly, Bui and Kelly[51] reported that 32 of 52 *cldns* found in the spotted green puffer fish (*Tetraodon nigroviridis*) were present in gill tissue. Using a primary cultured gill epithelium, Bui and Kelly[51] were also able to confirm that 29 of the 32 *cldns* found in gill tissue were present in the gill epithelium. Therefore it is unlikely that any of the aforementioned 29 *Tetraodon* cldns are exclusive to gill vascular tissue. The three *cldns* that were present in gill tissue but were absent in the primary cultured gill epithelium (*cldn-6, -10d* and *-10e*) were speculated to be missing because the culture was composed of only one gill epithelium cell type, the gill pavement cell (PVC).[52] The rationale for this suggestion was based on the observation that *cldn-6, -10d* and *-10e* are responsive to changes in external salinity.[52] It was therefore hypothesized that these *cldns* were present in another gill epithelium cell type, or more specifically, the gill epithelium ionocytes (e.g., mitochondria-rich cells, MRCs).[52] We are now able to accept this hypothesis as we have recently found that Cldn-6, -10d and -10e co-localize with Na^+-K^+-ATPase immunoreactive ionocytes in the gill epithelium of *Tetraodon* (Bui and Kelly unpublished observations) and abundant Na^+-K^+-ATPase is one of the hallmarks of gill ionocytes.

The relationship between the gill epithelium and the surrounding environment is intimate, and the physiological consequences of environmental change on the structure and function of the gill epithelium are striking.[45] Because changes in environmental conditions can be extremely varied, and diverse species of teleost fish cope with environmental change quite differently, the importance of *cldns* in the gill has already been addressed in a range of teleost species. These include broadly used model organisms such as zebrafish[53] and *Tetraodon*,[51,52,54-56] as well as other models such as *Tetraodon biocellatus*,[57] *Salmo salar*,[58,59] *Oncorhynchus mykiss*,[21,60,61] *Fundulus heteroclitus*,[62] *Carassius auratus*[21,23] and *Paralichthys lethostigma*.[63] When taking into account the aforementioned species and others, the number of *cldns* reported in gill tissue of teleost fishes is at least 44 (for review see ref. 6). This is because some species express *cldns* in gill tissue that other species do not (see ref. 6). For example, *cldn-27c* is found in the gill tissue of *Tetraodon* but not *Fugu*, while *cldn-d* (= *cldn-29a*) is found in the gill tissue of zebrafish and *Carassius auratus*, but not in the gill tissue of *Fugu* or *Tetraodon*.[6]

Of the studies conducted to date on gill *cldns*, one of the most broadly considered areas relates to the role these proteins may play in the "tight" freshwater (FW) fish gill vs. "leaky" SW fish gill paradigm which is associated with basic strategies of teleost fish osmoregulation. This paradigm helps to explain how passive paracellular ion loss is held in check when fishes are hyperosmoregulating in a hyposmotic (i.e., FW) environment and how paracellular Na^+ secretion can occur across the gill epithelium of a hypoosmoregulating fish in a hyperosmotic (i.e., SW) environment (for review see ref. 45). Increased abundance of gill mRNA encoding for presumed barrier-forming *cldns* (e.g., *cldn-3* and *-8* isoforms) have been reported following SW to FW transfer or acclimation to hyposmotic conditions (e.g., FW and ion-poor FW) where

paracellular ion loss needs to be restricted.[23,54,55,57] Indeed, if fishes are acclimated to ion-poor FW (IPW), where ion levels are lower than typical FW and the ionic gradient between extracellular fluid and surrounding water is greater than that found in FW (i.e., an extreme hyposmotic environment), the abundance of presumed barrier forming *cldns* in the gill elevates even further.[23,57] In contrast, increased abundance of mRNA encoding for presumed pore forming *cldns* (e.g., *cldn-10* isoforms) have been noted in the gills of fishes in a hyperosmotic environment.[52,59] In the gills of a SW (or SW acclimated) teleost fish, shallow "leaky" junctions are found between MRCs and a gill cell type known as an accessory cell (AC). Na^+ is proposed to move through these "leaky" junctions down an electrochemical gradient from extracellular fluid to SW.[45] The absence of *cldn-10* isoforms in gill PVCs[51,52] and the presence of *cldn-10* isoforms in gill ionocytes (Bui and Kelly, unpublished observations) supports the notion that these *cldns* participate in the movement of Na^+ through "leaky" gill TJs.[51,52,59] However, despite evidence to suggest that select *cldns* in the gill epithelium of teleost fishes may function in a manner similar to their orthologs in mammals, functional studies are generally lacking. Furthermore, functional work will be particularly important for characterizing *cldns* that only appear to be found in teleost fishes. A first in this regard is the recent report by Engelund et al.[58] who showed that transfecting *Salmo salar cldn-30* into a mammalian kidney cell line decreased epithelial conductance and paracellular permeability to monovalent cations, thus confirming it as a barrier-forming protein.[58]

At the transcriptional level, a number of gill *cldns* are also reported to be sensitive to alterations in environmental pH or more specifically environmental acidification.[53] Long-term acclimation to low pH, for example, resulted in: (1) an increase in mRNA abundance of *cldn-a, -b* (= *-30d*), *-c* (= *-3d*), *-d* (= *-29a*), *-e* (= *-28b*), *-f* (= *-27b*), *-h* (= *-3a*), *-j* (= *-6*), *-7* and *-12,* (2) a decrease in mRNA abundance of *cldn-2* and *-8* and (3) both an increase and decrease in mRNA abundance of *cldn-g* (= *-31*) and *-i* (= *-32a*) (at different points during acclimation).[53] However, despite the changes in *cldn* levels observed, the authors concluded that cation (Na^+) balance in low pH surroundings was primarily maintained in zebrafish by increasing Na^+ uptake rather than reducing paracellular Na^+ loss, because paracellular permeability appeared to remain elevated through the duration of acid water exposure.[53]

A final comment on *cldns* in the gill epithelium of teleost fishes is their responsiveness to endocrine factors involved in the regulation of salt and water balance in these organisms. Again, the majority of observations have been made at the transcriptional level,[21,22,52,60,61,64] and in some cases these observations have been causally linked to measured changes in gill epithelium paracellular permeability,[21,60,61] but the effects of corticosteroids in particular are striking. There is also evidence to suggest that in rainbow trout PVCs, select *cldns* may be responsive to the actions of cortisol (the principal corticosteroid in teleost fishes) through either the mineralocorticoid receptor (i.e., *cldn-28b* and *-30*) or glucocorticoid receptor (i.e., *cldn-3a*), while others respond to cortisol treatment through both receptors.[61] In addition to observations of corticosteroid effects in the gill and gill epithelium of teleost fishes, the mRNA abundance of *cldn-28a* has been reported to increase in gill tissue following systemic prolactin injections in SW-acclimated Atlantic salmon.[64]

Claudins in the heart and circulatory system of teleosts

The cardiovascular system of teleost fishes is a closed system that consists of a network of branchial (gill) and systemic blood vessels and capillaries connected to a two-chambered heart serving as a pump.[65] Comparatively few *cldns* have been associated with the cardiovascular system of fishes. Hematopoietic and endothelial cells of zebrafish have been reported to express *cldn-g* (= *cldn-31*),[66] and in zebrafish embryo *cloche* mutants that exhibit impaired development of hematopoietic and endothelial cell lineages, *cldn-g* is shown to be downregulated or absent.[66,67] Thus, *cldn-g* is speculated to be involved in cell adhesion during zebrafish erythropoiesis. In the vascular cord of developing zebrafish, *cldn-5* has been detected in the cell-cell contacts of arterial but not venous endothelial cells.[68]

In addition to the aforementioned proteins, a number of *cldns* are reported to be expressed in the heart tissue of teleosts, but the function in this tissue is currently unknown. For example, in the *Fugu* heart, mRNA encoding for *cldn -2, -3a, -3b, -6, -7a, -7b, -8c, -11a, -12, -14b, -20a, -23a, -26, -28a, -28c, -30a, -30b, -30c, -30d, -31* and *-32b* have been reported (Loh et al. 2004; see **Table 1**) and in the zebrafish, *cldn -b, -i, -1, -7, -10, -11 and -12* were reported to be found in the heart (see **Table 1**).[53,69]

Claudins in the teleost reproductive system

Teleost fishes employ a variety of reproductive strategies including oviparity, ovoviviparity and viviparity. Oviparous or ovoviviparous females usually have a pair of ovaries, consisting of follicles at different maturation states, while males have a pair of testes.[65] In *Fugu*, only 8 *cldns* have been reported in ovarian tissue, while 15 were found in testes.[45] In contrast, at least 18 *cldns* have been found in zebrafish ovarian tissue, while 12 were found in zebrafish testis.[69] The marked difference in ovarian tissue has been suggested to result from the different breeding patterns of zebrafish and *Fugu*.[69] Specifically, laboratory zebrafish can breed throughout the year and their ovaries contain follicles at all developmental stages.[69] This breeding strategy contrasts with the one found in *Fugu*, which has been described as a spring breeding fish in the wild.[70] Therefore in contrast to the asynchronous ovary of zebrafish, *Fugu* has a synchronous ovary where the oocytes, save a few residual pre-vitellogenic follicles, would all undergo maturation during the spring.[70,71]

The role of *cldns* in teleost gonads has been studied primarily in the context of gametogenesis, leading up to the release of gametes necessary for fertilization. This process is similar to an epithelial-mesenchymal transition (EMT) of other vertebrates, where cells become detached from confluent tissue and mobile. The ovarian follicles of zebrafish are reported to have TJs throughout their development, but in late stages (e.g., mature follicles) TJ architecture is less distinct relative to early stage pre-vitellogenic follicles.[72] In zebrafish ovarian tissue, *cldn* transcript abundance varies by four orders of magnitude, and two of the most abundant *cldns*, *cldn-g* (= *cldn-31*) and *-d* (= *cldn-29a*), along with at least four others, exhibited a decrease in abundance as follicle development progressed.[69] The changes observed in *cldn* abundance were

suggested to play a role in the loss of TJ definition as described by Kessel et al.[72] and a remodeling of somatic layer TJs during zebrafish folliculogenesis.[69] Taken together, changes in the integrity of the TJ complex of zebrafish follicles during maturation were ultimately suggested to play a role in ovulation.[69] It is also noteworthy that in a follow-up study, it was reported that the mRNA abundance of *cldn-g* was decreased by GDF9 in mid-vitellogenic zebrafish ovarian follicles.[73] GDF9, a growth factor thought to be involved in follicle development, is present in zebrafish primary ovarian follicles at high levels, but declines as folliculogenesis progresses.[73]

Claudins in the gastrointestinal tract of teleosts

In *Fugu*, 24 *cldns* have been reported in the GI tract (Loh et al. 2004) and up to 30 members of the *cldn* family have been described in the GI tract of teleost fishes examined thus far (see **Table 1**).[28,29,45,53-55,69,74-78] Despite this, we know very little about the role these proteins play in the GI tract physiology of teleost fishes. It has been reported that the abundance of select *cldns* can vary spatially along the GI tract,[22,69,76,78] and this is in line with the spatial variation of GI tract *cldns* in other vertebrates (e.g., refs. 79–80).

The GI tract of teleost fishes plays an important role in the regulation of salt and water balance.[81] In a hyperosmotic environment (i.e., SW) where teleost fishes are presented with the problem of tissue dehydration, the GI tract contributes to the acquisition of water and to the elimination of excess salts. Teleosts achieve this by drinking and desalinating SW (i.e., moving monovalent ions from the gut lumen to extracellular fluid) in the anterior regions of the GI tract through active and passive transport processes. As ions move from the gut lumen to extracellular fluid, water follows by osmosis and excess monovalent ions in the blood are then secreted across the gill epithelium. However, since water is being removed and divalent ions that are abundant in SW (e.g., Mg^{2+} and Ca^{2+}) remain, the contents of intestinal fluids become increasingly concentrated as they move toward posterior gut regions. This is ultimately dealt with by precipitation and rectal secretion of divalent ions as insoluble carbonates.[81-83] In association with anterior to posterior changes in GI tract luminal content, the GI tract of teleost fishes has been reported to progressively "tighten,"[84] thus preventing leakage of water back into the gut lumen. Transcript encoding for presumed barrier forming *cldn-3d* has been reported to progressively increase from the anterior to posterior regions of the GI tract of *Tetraodon* acclimated to SW, but not in FW acclimated fish.[75] Transcript abundance of *cldn-3a* is also higher in the hindgut of SW- vs. FW-acclimated *Tetraodon* while *cldn-3d* mRNA is lower in the anterior GI tract of SW-acclimated *Tetraodon* vs. those in FW.[75] In contrast, SW did not induce any alteration in the mRNA abundance of *cldn-3a*, *-3b* or *-3c* in the intestine of *Salmo salar*.[77] However, *cldn-25b* exhibits a progressive increase in mRNA abundance along the GI tract of *Salmo salar*.[76] Due to the sequence similarity of *cldn-25b* with barrier forming mammalian CLDN-4, increased GI tract *cldn-25b* mRNA abundance in SW *Salmo salar* was suggested to be involved in "tightening" the intestinal epithelium.[76] However, the *Salmo salar* intestine also exhibited increased *cldn-15* mRNA abundance in response to SW acclimation and *cldn-15* is suggested to be pore-forming.[76] Nevertheless, different alterations in the mRNA abundance of *Salmo salar cldn-15* and *cldn-25b* were found following injections of osmoregulatory hormones, supporting the idea of different functions for these proteins in the intestine.[76] It should also be noted that a progressive increase in *cldn* mRNA abundance along the GI tract has been found in stenohaline FW teleost fishes.[22,78] These fishes would never experience SW, therefore it seems possible that common functional themes may be found for GI tract *cldns* in fishes that are independent of environment and may be linked to similarities in chyme processing.

The deleterious effects of pathogens on intestinal TJ integrity, as determined by morphological changes, have been reported in fishes such as *Salmo salar* and *Oncorhynchus mykiss* (e.g., refs. 85–87). More recently, the effect of pathogen presence on *cldn* transcript abundance in the intestine of *Cyprinus carpio* has also been reported.[78] Following cyprinid herpesvirus 3 (CyHV-3) infection mRNA encoding for *cldn-2, -3c, -11* and *-23* significantly elevated in the intestine of *Cyprinus carpio* in conjunction with an upregulation of mRNA encoding for genes involved in the inflammatory response.[78] It was proposed that alterations in *cldn* abundance may contribute to mechanisms that compensate for a possible disruption of proteins by nitric oxide produced during an immune response of the host to virus-induced tissue damage.[78]

Even though at least 30 *cldns* have been reported to be present in the GI tracts of teleost fishes studied so far (see **Table 1**), only a small fraction of them have been examined to date and almost no functional studies have been conducted. Considering the importance and complexity of the teleost fish GI tract, the role of Cldns in this tissue will be an exciting area for future study.

Claudins in the kidney of teleosts

The teleost fish kidney assists in the maintenance of homeostasis by contributing to the elimination or retention of excess water and reabsorption or secretion of ions. Like the mammalian kidney, the nephron of the fish kidney can be separated into functionally distinct segments: the glomerulus, proximal tubule, distal tubule and collecting duct.[81] Between FW and SW fishes however, the length and physiological function of these segments may differ. In the FW fish nephron for example, the distal tubule (or "diluting segment"), which is characterized as "tight" and relatively water impermeable, selectively reabsorbs a significant amount of Na^+ and Cl^-, thus resulting in large amounts of relatively dilute urine.[81,88] Marine fishes however exhibit varying levels of structural or functional degeneration of distal nephron regions and may lack a distal segment of the nephron altogether.[81,88] Instead, the proximal segments of the SW fish nephron, which are characterized by low TER and high permeability, initially facilitate ion secretion (e.g., Na^+, Cl^-, Mg^{2+}, SO_4^{2-}), followed by Na^+, Cl^- and water reabsorption in the later proximal segments to prevent dehydration.[81] Despite these differences in physiological function between the nephron segments of FW and SW teleosts, the general permeability trend along the teleost fish nephron is analogous to that of the mammalian nephron. That is a trend of decreasing permeability from the "leaky" proximal tubule to the "tight" collecting duct.[89,90] Given that numerous CLDNs are expressed in a segment-specific manner in the mammalian kidney, these proteins have been identified as

major determinants in the permeability profiles between the different nephron segments.[91]

Taking together functional similarities between the mammalian and teleost fish nephrons and the fact that to date, over 35 *cldns* have been reported in the kidney of various teleost fishes (see **Table 1**), it is likely that the segment-specific barrier properties of the fish nephron may also be dictated in part by the differential expression of *cldns* among distinct renal segments. However, until recently the only study that demonstrated spatial differences in the distribution of a TJ protein along the teleost fish nephron was for occludin.[92] Recently, however, it has been reported that *cldn-b* (= *cldn-30d*) in the zebrafish nephron exhibits far greater abundance in the collecting tubule vs. the proximal tubule.[93] In addition, earlier reports that examined renal *cldn* abundance in response to salinity variation alluded to segment-specific claudin expression patterns. In *Tetraodon* for example, mRNA abundance of *cldn-3a, -3b, -3c* and *cldn-8a, -8b, -8c, -8d* and renal Na^+-K^+-ATPase activity was shown to be significantly reduced following acclimation to a hyperosmotic environment.[54,55] Because Na^+-K^+-ATPase is abundant in the distal tubule of the fish nephron (e.g., see ref. 92), it was suggested that *cldn-3* and *-8* isoforms may be more abundant in the distal tubule of the fish nephron and that the reduced mRNA abundance of these *cldns* and reduced Na^+-K^+-ATPase activity may reflect the degeneration of renal distal segments in response to increased environmental salinity.[54,55] This proposed expression pattern in the fish nephron is consistent with the expression of barrier-forming CLDN-3 and -8 within the "tighter" distal regions of the mammalian nephron.[91] Further support for this idea was provided by an additional study in another species of *Tetraodon* (*T. biocellatus*), where significant reductions in *cldn-3a, -3c, -3d* and *cldn-8a* and *-8c* mRNA levels were reported in renal tissue following SW acclimation, alongside a general decline in distal tubule surface area and number, as well as distal tubule TJ depth.[57] Accordingly, when *T. biocellatus* were acclimated to IPW, where salt reabsorption and water elimination by a "tight" distal tubule becomes increasingly important, *cldn-3a, 3c, -3d* and *cldn-8a, -8b, -8c* and *-8d* mRNA abundance was significantly elevated relative to SW-acclimated fish.[57] These changes in *cldn* abundance in response to IPW acclimation were accompanied by a significant increase in distal tubule surface area and distal tubule and collecting duct TJ depth, in addition to a significant reduction in proximal tubule numbers and surface area.[57] Correspondingly, renal *cldn-3b* mRNA abundance in *T. biocellatus*, which remained unchanged by SW-acclimation, was significantly reduced by IPW-acclimation, suggesting this *cldn-3* isoform may be associated with the proximal tubule.[57]

Species-specific differences however in renal *cldn* expression following salinity variation have been noted. For example, *cldn-3b* levels were significantly reduced in renal tissue of *T. nigroviridis* following acclimation to SW or hypersaline SW,[54] while in the Atlantic salmon (*Salmo salar*), *cldn-3b* and *-3c* were significantly elevated in the kidney following transfer from FW to SW.[77] These variations in the response of *cldns* within teleost fish kidney tissues may also be time-dependent. For instance, in juvenile *Salmo salar*, renal *cldn-3a* and *-3b* were only transiently elevated during the early months of smoltification.[77]

Claudins in the skin of teleosts

Similar to the gill epithelium, the epidermis of teleost fishes is a large epithelial surface area that directly interfaces with surrounding water.[81] However, unlike the gill epithelium, which plays a particularly dynamic role in the maintenance of homeostasis in teleost fishes (see section on Claudins in the teleost gill), the integument of most adult teleost fishes is generally thought to act as a simple barrier to solute movement. Nevertheless, the skin does play an important role in this regard, and among other things, it also plays an important role in defense against pathogen invasion. The epidermis of teleost fishes arises from a stratum germinativum which adheres to a basal lamina that tightly links the epidermis and dermis. However, unlike the tetrapod epidermis, the epidermis of teleost fishes is not keratinized and lacks a stratum corneum. Instead, the teleost epidermis consists of a thin layer of stratified squamous epithelial cells which are covered by a mucous cuticle.[94,95]

In the skin of *Fugu*, 25 *cldns* were found to be expressed by Loh et al.[45] In addition, 10 *cldns* (*cldn-3a, -3c, -8a, -8b, -8c, -8d, -27a, -27b, -27c* and *-27d*) have been reported in the skin of *Tetraodon*,[54,55] 6 (*cldn-b, -e, -i, -1, -8* and *-11*) in the skin of zebrafish,[69] 7 (*cldn-b, -d, -e, -h, -7, -8d* and *-12*) in goldfish skin[22] and 7 (*cldn-2, -3b, -3c, -7, -11, -23* and *-30*) in common carp skin[78] (see **Table 1**). However, even though a large number of *cldns* have been reported in teleost fish skin, little attention has been paid to their potential role(s) in this tissue and almost nothing is known about how they are distributed within the integument.

It has been proposed that changes in skin *cldn* mRNA abundance in *Tetraodon* acclimated to SW vs. those in FW may reflect changes in the barrier properties of teleost fish skin in response to environmental change.[54,55] This is supported by an observed increase in the mRNA abundance of putative barrier forming *cldn-3a, -3c, -8c, -27a* and *-27c* in the skin tissue of fish acclimated to hyperosmotic environments.[54,55] In fishes, the notion that Cldns may be involved in the regulation of epidermal permeability following changes in environmental conditions is further strengthened by recent observations that in *Tetraodon* skin, Cldn-6 is co-localized with Na^+-K^+-ATPase-immunoreactive cells and its abundance is also sensitive to changes in environmental salt concentration (Bui and Kelly, unpublished observation). In addition, putative pore-forming Cldn-10d and -10e are also present in *Tetraodon* epidermis, although their potential role(s) in this tissue remains elusive (Bui and Kelly, unpublished observation).

In the common carp, exposure to CyHV-3 caused a reduction in skin *cldn-23* and *-30* mRNA abundance, along with decreased abundance of other molecules involved in skin defense (e.g., mucin 5b and β defensin).[96] Failure to elicit an immunogenic response further suggested that downregulated *cldn* abundance may help the virus gain access to deeper tissue. As a consequence, a defective epidermal barrier layer caused by a reduction in mucus and Cldn abundance is proposed to promote secondary infection and facilitate viral spread.[96] Cldns are also present in the mechanosensory organs (e.g., neuromasts) found in the skin of

teleost fishes.[97,98] In zebrafish, *cldn-b* (= *cldn-30d*) is prominently expressed around the periphery of peridermal cells and is involved in the development of the lateral line system.[12,98]

Conclusion and Perspectives

It has been 13 y since the first *cldn* was reported in a teleost fish, and we are now aware of ~63 *cldns* in 16 teleost species studied so far (see **Table 1**). A major obstacle in our understanding of *cldns* in teleosts is undoubtedly the sheer number of them. For example, there are more *cldns* in the gill of a teleost fish (e.g., 32 in *Fugu*) than there are in mammals (~27 described so far, see ref. 10) and to date, *cldns* have only been fully enumerated and described in one species of teleost, *Takifugu* (= *Fugu*) *rubripes*.[45] In this regard, an initial challenge for any program interested in teleost fish *cldn* function may be the problem of enumeration so that screening can reveal specific *cldns* that might be of importance (and therefore of interest) in a system under examination. In the absence of this, solid hypothesis driven work that specifically targets a select *cldn* (or *cldns*) can reveal a great deal about their function in fishes, as is evident in some of the elegant work already conducted in this area. Moving forward, it seems very likely that the zebrafish model will play a major role in how our understanding of *cldns* in teleost fishes (and in vertebrates as a whole) will develop. Indeed, there are already almost as many studies published on zebrafish *cldns* as there are on *cldns* in all other teleost fishes combined (see **Table 1**). The zebrafish model has and continues to provide answers about *cldn* function during development, morphogenesis and organogenesis. It is also likely that the zebrafish model will contribute significantly to how we understand the role of specific *cldns* in organ systems once they are developed. In all cases, this will allow insight into zebrafish modeled aspects of *cldn* function that may be relevant to human health and disease. In addition, the zebrafish is a popular model in many other realms where *cldns* may become a focus of attention, such as toxicology, environmental sciences or evolutionary theory to name a few.[100-103] However, from a comparative standpoint, it is also important to remember that the diversity of the teleost group and the large numbers of *cldns* found therein, strongly suggests that an additional wealth of information on *cldn* function can be found by looking at diverse species. Indeed, if we take this a step further and include other fishes, the possibilities for unique insight will become even greater. For example, *cldn* characteristics, and the role that *cldns* may play in the distinctive biology of some extant Agnathans, such as members of the Myxini (i.e., hagfish), would be very interesting. These osmoconforming fishes have blood plasma that is essentially the same composition as SW (for review see ref. 99). However, deep multi-stranded TJs are present in tissues of agnathans such as the gill epithelium (for review see ref. 6). In addition, nothing is known about TJ proteins in Chondrichthyes (e.g., sharks). These animals also have an interesting and unusual biology in that they possess high circulating and tissue levels of urea (~350 mM), which is used as an organic osmolyte. Yet despite a large urea concentration gradient between blood and water, urea is retained very effectively. There is no doubt that this is achieved in part by reduced transcellular urea permeability, but whether TJ proteins such as *cldns* play a role in reducing urea permeability through the paracellular pathway has yet to be considered. Taken together, the future for *cldn* research in fishes is very bright indeed.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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