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Gene expression pattern of *Claudin-1* during chick embryogenesis

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Abstract

Claudins are a family of proteins that are localized to tight junctions at the apical surface of epithelial cell layers. Over 24 family members have been identified in vertebrates. Despite being well-studied with respect to their function in tight junction selectivity and permeability, the embryonic expression patterns of most claudin family members have not been thoroughly investigated. Here, we report the cloning and expression pattern of a novel chick claudin family member that is most closely related to human claudin-1. Chick *claudin-1* was expressed throughout the ectoderm of stage 4–6 chick embryos. *Claudin-1* expression was particularly high in the neural epithelium and open neural tube, but decreased as the neural tube closed. High levels of *claudin-1* expression were also observed in the developing otic vesicle, nasal placode, ectodermal component of the pharyngeal arches, and in the apical ectodermal ridge of the limb bud from stage 17 onwards. *Claudin-1* expression was also detected in scleral papillae, feather buds and migrating primordial germ cells. Lower levels of *claudin-1* expression were observed in the endoderm, the ventral pharynx, and several of its derivatives including the bronchi, developing lung epithelium, esophagus, and gut. *Claudin-1* expression was detected in the nephric duct and the mesonephros, which are epithelialized derivatives of the intermediate mesoderm, but not in any other mesodermal derivates, including the heart, somites and developing muscle. With the exception of the migrating primordial germ cells and the primitive streak, all other tissues that expressed significant levels of *claudin-1* were epithelialized.

Keywords: Claudin-1; Chick embryo; Early development; Expression pattern; Epithelia; Feather buds; Neural ectoderm; Neural folds; Migrating primordial germ cells; Bronchi; Pharyngeal arch; Otic vesicle; Nephric duct; Scleral papillae

1. Results and discussion

1.1. Chick claudin-1

Tight junctions are located at the apical side of epithelial cells and form an anastomosing network of cell–cell adhesion sites that is critical for regulating paracellular diffusion and for restricting apical-basolateral transport of small molecules (reviewed in Tsukita and Furuse, 2002; Tsukita et al., 2001). Claudin molecules contain four transmembrane domains and are an integral part of the transmembrane component of tight junctions. The C-terminus of the claudin protein localizes to the cytoplasmic plaque of the tight junction that also contains a number of other protein components that link the membrane to the actin cytoskeleton (Matter and Balda, 2003). The specific claudin molecule present in the tight junction is an important determinant of the selectivity and permeability of the tight junction (Turksen and Troy, 2004).

We have isolated a full-length cDNA clone encoding the chick homologue of claudin-1. The chick *claudin-1* cDNA encodes a 211 amino acid protein (Fig. 1). Chick claudin-1 is 73% identical to the human and 70% identical to the mouse claudin-1 proteins (Furuse et al., 1998); the human and mouse claudin-1 proteins are 90% identical. Amino acids 2–23 of chick claudin-1 are 100% identical to a 22 amino acid peptide sequence for chick claudin-1 that was previously reported (Furuse et al., 1998). It is significantly less similar to other cloned chick claudin family members; sharing only 43% identity with chick claudin-3 and -5.

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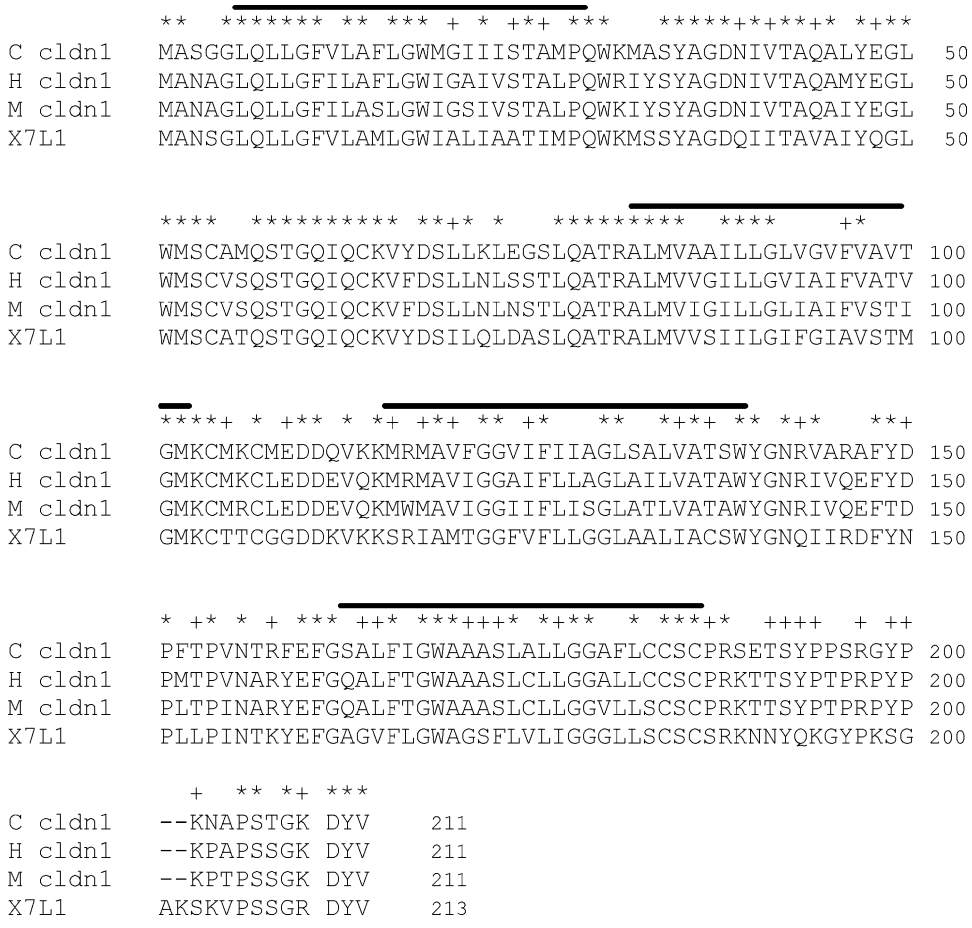

Fig. 1. Comparison of chick claudin-1 to other claudin family members. Alignment of the deduced amino acid sequence for the chicken *claudin-1* gene. Identities in all claudin-1 homologues are indicated by the asterisk and identities limited to the chick, human and mouse homologues are indicated by a '+'. The solid lines indicate the transmembrane domains.

The closest *Xenopus* claudin family member is X7L1, which is 58% identical to chick *claudin-1* (Fujita et al., 2002).

1.2. Claudin-1 expression in early chick embryos

We have used whole-mount in situ hybridization to examine the expression pattern of *claudin-1* mRNA in stage 4 to 34 (~1–8 days) chick embryos. *Claudin-1* mRNA was expressed in the area opaca and ectoderm of stage 4 embryos (Fig. 2a) and continued to be expressed at relatively high levels in the surface ectoderm for several days. Beginning at stage 6 (Fig. 2b), *claudin-1* expression increased in the thickening neuroepithelium. Expression of *claudin-1* remained high in the open neural folds, but dramatically decreased as the neural folds came together to form a closed neural tube (Fig. 2g–l). This decrease in *claudin-1* expression was observed first in the anterior neuroepithelium and progressed posteriorly, corresponding to the progression of neural tube closure (Fig. 2c–f). Immediately prior to closure, there was a ventral (low) to dorsal (high) gradient of *claudin-1* expression in the forming neural tube (Fig. 2h,m) and only very low levels of expression were observed in the closed neural tube (Fig. 2g).

At stage 9, *claudin-1* was expressed throughout the endoderm at the anterior end of the embryo (Fig. 2g). However, in more posterior regions of the embryo, *claudin-1* expression was observed only in the lateral endoderm (Fig. 2i,j) and not in the endoderm underlying the midline (Fig. 2h–l). *Claudin-1* was also expressed in the floor of the pharynx (Fig. 2m).

Lower levels of *claudin-1* expression were evident in the primitive streak than in the surface ectoderm, suggesting that *claudin-1* expression is downregulated as cells leave the ectoderm and ingress through the primitive streak. Between stages 6 and 13 no expression was ever observed in the mesoderm or its derivatives, including the developing heart tube, notochord, somites, and lateral plate mesoderm (Figs. 2,3).

1.3. Claudin-1 expression in 3- to 8-day chick embryos

At later stages of development, *claudin-1* expression was primarily observed in specific regions of thickened epithelia (Figs. 3–5). These included the nasal placode